Report

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Novel aspects of fatty acids: nutrition and biological functions
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Dietary lipids have been in focus since the very beginning of nutrition research, and recommendations have emphasized their effects related to blood cholesterol levels and other risk factors for cardiovascular disease. Progress in recent years has shown that fatty acids and specific lipid compounds have many other cellular and metabolic functions of key importance for normal cell metabolism, as well as in the development of obesity and related chronic diseases. Lipid components are important regulators of gene expression, cell signalling, inflammation and carcinogenesis, and also include anticarcinogenic factors. Epigenetic regulation and early metabolic programming is an evolving area where fatty acids seem to play a key role, and some lipid components are crucial for the development and function of the brain.

The symposium included plenary presentations and poster presentations of the host of newly discovered key functions of fatty acids and specific lipid components, and future challenges for research in this area were highlighted. The implications of these new developments for nutrition recommendations at various ages were discussed, along with the challenges and possibilities for the food industry to implement new findings into the development of nutritionally optimized foods and products with specific health-promoting effects.

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A summary report is given here based on the plenary presentations, which are published as Supplement 2/2006 of the Scandinavian Journal of Food and Nutrition, provided with this issue (1).

Introduction

An introductory presentation by *Bengt Vessby* entitled "Fat research and human health – at present and in the future" set the scene. The composition of fatty acids in the diet is reflected in circulating lipids, storage fat, membranes and intracellular lipids, and thereby influences a host of physiological functions. The leading cause of disease and premature death in developed countries, and increasingly in countries in transition, is still atherosclerotic diseases, with a well-established role of certain fatty acids that can be modified by dietary means. Today, we have a much more detailed knowledge of the physiological role of fatty acids and other lipids, with increasing understanding of mechanisms on the molecular level.

The following areas were mentioned as particularly challenging for future research: further exploration of physiological and pathophysiological effects of specific fatty acids, including differential effects of various saturated fatty acids; the importance of the amount (proportion of dietary fat) in human diets; effects of minor lipid components, both fatty acids and other lipids; gene–nutrient interactions, including genetically different sensitivity to effects of dietary lipids; and the interaction between fat intake and physical activity. Ectopic fat accumulation in the liver, pancreas and skeletal muscle was mentioned as one specific emerging cause of lipid-induced metabolic disturbances. More dietary intervention studies are needed to confirm hypotheses based on observational/epidemiological studies.

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Gene regulation, cell signalling and obesity

In the first session on gene regulation, cell signalling and obesity, *Donald Jump* took a starting point on how the type and quantity of dietary fat ingested contribute to the onset and progression of chronic diseases, such as diabetes and atherosclerosis. The liver plays a central role in whole-body lipid metabolism and responds rapidly to changes in dietary fat composition. In rodents, n-3 polyunsaturated fatty acids (n-3 PUFAs) enhance hepatic fatty acid oxidation and inhibit fatty acid synthesis and very low-density lipoprotein (VLDL) secretion, in part by regulating key transcription factors, such as peroxisome proliferator activated receptor-α (PPAR-α) and sterol regulatory element binding protein-1 (SREBP-1). These transcription factors control the expression of multiple genes involved in lipid oxidation and synthesis.

Jump further described that in humans, n-3 PUFAs also suppress VLDL secretion and blood triglycerides (TGs). This effect, however, may be more dependent on n-3 PUFA effects on hepatic lipid synthesis and VLDL secretion than on hepatic PPAR-α activity and peroxisomal fatty acid oxidation. Docosahexaenoic acid (DHA, 22:6 n-3) is the only n-3 PUFA accumulating in livers of rodents or humans ingesting essential fatty acid-sufficient or n-3 PUFA-enriched diets. He summarized that n-3 PUFAs affect the phosphorylation status of several insulin-regulated signalling pathways.

Richard Deckelbaum focused on the fact that biologically favourable effects of eicosapentaenoic acid (EPA) and DHA, such as reduction of risk factors associated with cardiovascular diseases, diabetes and cancer, are likely to be mediated through effects on several distinct pathways within cells, tissues and organs. These include direct interactions in changing the composition of cell membranes and membrane function, activating or suppressing signalling molecules, interacting directly with DNA as well as with proteins that affect the processing of transcription factors. It was also recently suggested that because of their high content of double bonds, rather than being pro-oxidant, EPA and DHA may act as scavengers of reactive oxygen species. Some of the effects of EPA and DHA may not be directly related to the fatty acid molecule itself but rather to their metabolites, such as eicosanoids or other molecules. Mechanisms underlying the specific and sometimes different responses by cells to EPA and DHA need to be better understood so that we can anticipate better selectivity of DHA compared with EPA, not only in modulating key biological pathways and gene expression, but also in preventing and treating specific diseases.

Gérard Ailhaud stated that recent evidence from animal and human studies favours the possibility that changes in the balance of essential PUFAs alter the early stages of adipose tissue development, not only during foetal life and infancy, but also later in life. In both rodents and humans, long-chain fatty acids act at the preadipocyte stage and trigger the formation of adipocytes.

Experiments with wild-type (WT) mice and mice invalidated for the cell-surface prostacyclin receptor ($ip^{-/-}$ mice), under isoenergetic conditions, have shown that pups from WT mothers fed a linoleic acid (LA, 18:2 n-6) diet are 40% heavier 1 week after weaning than those from mothers fed a LA/linolenic acid (LNA, 18:3 n-3) diet, and that the weight difference between mice fed LA and those fed LA/LNA is maintained at the adult age. Furthermore, the LA-induced enhancement of fat mass is abolished in $ip^{-/-}$ mice, illustrating the critical role of AA (20:4 n-6) and the prostacyclin receptor in excessive adipose tissue development.

According to Ailhaud, changes observed over the past few decades in the fatty acid composition of dietary fats in breast milk and formula milk, i.e. a large increase in LA with slight or no change in LNA content, may be at least partly responsible for the dramatic rise in the prevalence of childhood overweight and obesity, acting in concert with a positive energy balance.

Growth and development/epigenetics

Robert Waterland introduced his presentation by referring to human epidemiological data indicating that nutrition during prenatal and early postnatal development can affect susceptibility to various chronic diseases in adulthood (the developmental origins hypothesis). Controlled studies in animal models corroborate that nutritional exposures during critical periods of development can yield lasting influences on gene expression and metabolism, but our understanding of the fundamental biological mechanisms underlying such phenomena remains rudimentary.

Nutritional influences on the developmental establishment of epigenetic gene regulatory mechanisms could link early nutrition to adult chronic

disease susceptibility. Just as genetic variation contributes to individual susceptibility to chronic disease, it is increasingly evident that so too does individual epigenetic variation.

Dietary fatty acids could affect the establishment of epigenetic mechanisms by stimulating transcription of specific genes during critical developmental windows. For example, the PPAR-α transcription factor is activated by a variety of fatty acids to transactivate specific genes. Transcriptional activation impedes epigenetic silencing. Recent data indicate that lipids and lipoprotein components interact directly with chromatin structure to influence gene expression. Hence, intake of specific dietary fatty acids during development could persistently affect gene expression by altering the establishment of epigenetic mechanisms.

Birgitta Strandvik emphasized that several papers have reported a different fatty acid profile in phospholipids and adipose tissue in obese individuals, suggesting that the changing ratio of fatty acids in human diets during the past 30 years might have an influence on the rapidly increasing prevalence of welfare diseases. Essential fatty acids and the ratio between the n-6 and n-3 series are important factors behind programming in experimental models. Modifying essential fatty acids in the diet of the pregnant and lactating rat, but not thereafter, have resulted in defective immunological development of oral tolerance, defective bone mineral density and symptoms indicating metabolic syndrome in the adult offspring. One mechanism may be the involvement of the leptin system, which was severely disturbed in the neonatal period.

In a prospective study of healthy 4-year-old children, lower fat and higher sucrose contents of the diet were associated with higher body mass index and higher fat mass, and influenced insulin homeostasis, as measured by fasting insulin concentration and HOMA (homeostatic model assessment) indices. This influence was related to the growth rate from birth to 4 years of age, especially in girls. Obese children had significantly lower n-3 fatty acids and the concentrations correlated negatively with both the amount of adipose tissue and the HOMA β-cell index. Strandvik concluded that the marked change worldwide in the ratio of the essential fatty acids in human diets during recent decades may be associated with the increase in the welfare diseases by metabolic programming.

Ricardo Uauy addressed the importance of dietary lipids for the brain during development and ageing. The brain and retina are rich in long-chain polyunsaturated fatty acids (LCPs), and DHA in particular has been shown to affect retinal and brain development in humans. Provision of n-3 LCPs in preterm and term babies is associated with enhanced retinal electrical responses to light stimuli and to a pattern of brain cortex-related visual acuity maturation that is similar to that observed in human milk-fed infants. Some results in young children suggest that neurodevelopment and cognitive abilities are also enhanced by early provision of n-3 LCPs.

Animal studies have demonstrated that ageing is associated with decreased levels of DHA in the brain. A drop in n-3 LCP level in total brain lipids has also been reported for humans, postulating that this decline is correlated in part with age-related deterioration of central nervous system function. Evidence supporting the potential importance of n-3 LCP from fish consumption for good cognitive health in older age is beginning to emerge. The n-3 LCPs inhibit hepatic TG synthesis and modulate eicosanoid function, and induce vascular relaxation, diminished inflammatory processes and decreased platelet aggregation. These actions may be important in decreasing brain microinfarcts, which are a cause of vascular cognitive impairment shown to be directly associated with cognitive decline. A large randomized controlled trial (RCT) is presently ongoing in a group of people aged over 70 years to assess whether a n-3 LCP supplement will preserve retinal function and prevent the age-related cognitive decline observed during ageing.

Sjurdur F. Olsen summarized present knowledge on dietary n-3 fatty acids and pregnancy outcomes, with an emphasis on evidence from observational and intervention studies in humans. Preterm birth, intrauterine growth retardation and pre-eclampsia are the most common complications of pregnancy, and account for a substantial proportion of perinatal deaths and the morbidity and developmental deficits seen during infancy. Consumption of n-3 fatty acids in pregnancy may prevent preterm delivery and delay timing of spontaneous delivery through an impact on eicosanoids involved in the initiation of parturition. Some animal experiments favour this possibility, whereas results from human observational studies and RCTs show conflicting results.

It has been suggested that a high intake of n-3 fatty acids increases foetal growth, but again the results are conflicting. It has also been suggested that increased intake of n-3 fatty acids may reduce the risk of, or ameliorate pre-eclampsia, but most studies have been unable to substantiate this.

Effects mediated in the intestine

Colin D. Funk introduced this section with a presentation on eicosanoid signalling. Eicosanoids are lipid mediators consisting of prostaglandins, leukotrienes, lipoxins and various epoxy-eicosatrienoic acids and epoxy-alcohols primarily derived from AA and to a lesser extent from EPA and dihomo-γ-linolenic acid. This large class of bioactive lipids is derived from the initial release of PUFAs from the *sn*-2 position of glycerophospholipids predominantly from cytosolic phospholipase A_2, and subsequent conversion by prostaglandin H synthases-1 and -2 [PGHS-1, PGHS-2; also known as cyclooxygenase-1 and -2 (Cox-1, Cox-2)], lipoxygenases or various members of the cytochrome P450 family. Eicosanoids control a vast array of physiological functions, including female reproductive function and parturition, platelet aggregation and vascular homeostasis, as well as renal function and roles in inflammation initiation and resolution.

Funk reported studies on the functions and signalling of eicosanoids, in particular the prostaglandin class of molecules, using a series of induced mutant mouse strains. Despite major decreases in thromboxane, pregnant females were able to maintain normal pregnancy and parturition. However, platelet function was severely curtailed. Other genetically manipulated mice (PGHS-2 Y385F mice) are more susceptible to thrombosis and blood pressure elevation compared with controls and an explanation for this phenotype can be explained by a relative decrease in vascular-derived prostacyclin formation. PGHS-2-derived eicosanoids are important for patency/closure of the foetal ductus arteriosus. Overall, these studies are providing new insights into eicosanoid signalling.

Elizabeth K. Lund gave an overview presentation of the epidemiological evidence and the potential mechanisms of action of dietary fatty acids in relation to colon cancer. Colorectal cancer develops over many years as a result of an accumulation of epigenetic and genetic changes that result in a loss of control of cell proliferation and a failure of damaged cells to undergo apoptosis. The rate of accumulation of these changes and the consequent risk of developing colorectal cancer are believed to be susceptible to a range of both carcinogenic and anticarcinogenic dietary components and, while recognized genetic factors and inflammatory bowel disease account for only about 10–15% of cases, environmental factors, particularly diet, are believed to be the dominant causal factor.

Intervention studies in both humans and experimental animals provide evidence of a potentially protective role for n-3 PUFAs, particularly the very long-chain forms found in fish. The n-3 PUFAs decrease cell proliferation *in vivo* and increase apoptosis both *in vivo* and *in vitro*. Mechanisms that could underlie potential protective effects include modification of gene expression via nuclear receptor signalling, including a down-regulation in Cox-2 expression, reduction in the production of proinflammatory prostaglandins, increase in the oxidation state of the cell, and functional changes to the cell and mitochondrial membranes.

Rui-Dong Duan focused on lipid signalling by phospholipids, particularly sphingomyelin (SM), and colonic carcinogenesis. Differing from other phospholipids, metabolism of SM by sphingomyelinase (SMase) and ceramidase (CDase) generates ceramide and sphingosine, which inhibit cell proliferation and induce apoptosis. In the intestinal tract, SM is sequentially hydrolysed by alkaline SMase (alk-SMase) and neutral ceramidase (N-CDase). Besides generating ceramide, alk-SMase can hydrolyse lysophosphatidylcholine and consequently reduce the formation of lysophosphatidic acid (LPA). Furthermore, alk-SMase can hydrolyse and inactivate platelet activating factor (PAF). Both LPA and PAF are potent stimulators of inflammation, proliferation and cancer metastasis in the colon.

In the past decade a special link between SM metabolism and colon cancer has emerged. Feeding animals with SM or ceramide inhibits the formation of colonic aberrant crypt foci and the development of colon cancer, which is associated with an accumulation of SM and reduction in ceramide in the tissues. Cell-culture studies showed that alk-SMase inhibits cell proliferation and DNA synthesis, accompanied by a reduction in SM and an increase in ceramide. In patients with colon cancer, alk-SMase activities in both mucosa and faeces are significantly decreased.

The expression of alk-SMase and N-CDase is subject to change by dietary factors. A high-fat diet significantly decreased the levels of alk-SMase. The water-soluble fibre from psyllium husk increased the expression of alk-SMase and reduced the N-CDase activity. Fish oil and AA in the diet have no effect. Alk-SMase can also be up-regulated by drugs with anti-inflammatory and anticancer properties, such as 5-aminosalicylic acid, ursodeoxycholic acid and ursolic acid.

Cell stress and inflammation

Philip C. Calder's presentation on LCPs and inflammation took its starting point in the opposing actions of n-6 and n-3 PUFAs. AA (20:4 n-6) gives rise to the eicosanoid family of inflammatory mediators (prostaglandins, leukotrienes and related metabolites). EPA and DHA act as an alternative substrate giving rise to mediators that are less potent than the analogues produced from AA. Furthermore, EPA and DHA give rise to newly discovered families of mediators termed E- and D-resolvins, respectively, which have anti-inflammatory and inflammation-resolving actions. Very long-chain (VLC) n-3 PUFAs also affect cell-signalling processes and gene expression in inflammatory cells, resulting in decreased expression of inflammatory cytokines (tumour necrosis factor, interleukin-1, etc.) and adhesion molecules, which may be important in protecting against acute and chronic inflammatory conditions. There is good evidence for the efficacy of VLC n-3 PUFAs in rheumatoid arthritis, with less strong evidence in Crohn's disease and rather weak evidence in asthma. The precursor n-3 PUFA, α-LNA, exerts some anti-inflammatory effects at very high intakes, perhaps reflecting the need for its conversion to EPA to be effective.

Patrick Schrauwen introduced his presentation on lipid-induced cell stress by highlighting that not only the white adipose tissue, but also peripheral tissues such as the liver, skeletal muscle and heart, have intracellular storage capacity for a limited amount of fatty acids. It is likely that these intracellular lipids serve as a rapid energy source during physical activity, for example. Indeed, endurance-trained athletes are characterized by increased amounts of intramyocellular lipids (IMCLs), which decline during endurance exercise.

However, the capability to store lipids in non-adipose tissues may have detrimental health effects in our sedentary Westernized society, where physical activity levels are low and obesity is becoming a major health problem. In this case, excess body fat is also excessively stored in liver, heart, pancreas and skeletal muscle. In these tissues, intracellular fat accumulation, in combination with a low oxidative capacity, is associated with decreased insulin sensitivity. Although the exact mechanism has not been completely unravelled, ample evidence suggests that fatty acid intermediates, such as diacylglycerol, fatty acyl-coenzyme A (CoA) and ceramides, can hamper insulin signalling. These fatty acid intermediates are likely to accumulate when a mismatch between fatty acid uptake and fat oxidative capacity exists; this explains why in the endurance-trained state, characterized by high fat oxidative capacity, high IMCL content does not lead to insulin resistance.

Reduced expression of transcription factors involved in regulation of oxidative genes, PPARs and PGC1, has been suggested to underlie the reduced oxidative capacity as observed in the (pre)-diabetic state. Patients with type 2 diabetes are characterized by increased lipid peroxidation, mitochondrial damage and reduced levels of the mitochondrial uncoupling protein UCP3, which protect mitochondria against lipid-induced oxidative damage. Thus, fatty acid accumulation in non-adipose tissues may precede the reduction in oxidative capacity and therefore be at the heart of type 2 diabetes mellitus.

Charles N. Serhan emphasized that a well-integrated inflammatory response and its ending, i.e. resolution, are essential in health and disease. It is important to achieve a complete understanding of the cellular and molecular events that govern resolution. Since the resolvins and protectins in animal models control the duration and magnitude of inflammation, mapping of these resolution circuits may provide new avenues for appreciating the molecular basis of many inflammatory diseases. The lecture gave an overview of recent advances regarding the biosynthesis and actions of the novel anti-inflammatory lipid mediators, resolvins, and protectins that are generated from the n-3 fatty acids (EPA and DHA). These lipid-derived mediators possess anti-inflammatory, proresolving and protective properties. Serhan concluded that defective resolution mechanism(s) may underlie our current appreciation of the inflammatory phenotype(s) that characterize some prevalent human diseases.

Effects on insulin and the metabolic syndrome

Geltrude Mingrone pinpointed the strong experimental evidence that changes in circulating non-esterified fatty acid (NEFA) levels are essential for stimulating insulin secretion. When the elevated levels of circulating NEFAs in 18–24 h fasted rats are acutely lowered by infusion of the antilipolytic agent nicotinic acid, subsequent glucose-stimulated insulin secretion is completely ablated, but it becomes supranormal when the NEFA concentration is kept high by coinfusion of a lipid emulsion plus heparin. Qualitatively similar results have been obtained in humans.

A source of fatty acid, either exogenous or endogenous, is necessary to support normal insulin secretion. β-Cell glucose sensitivity is impaired in obese subjects with type 2 diabetes and this is associated with the up-regulation of insulin secretion. The higher basal insulin secretory activity is related to a higher total stimulated insulin output as a consequence of the rise in the secretory system setpoint. Insulin sensitivity and insulin secretion correlate inversely. After malabsorptive bariatric surgery, circulating levels of NEFA decrease dramatically. This is associated with a significant improvement in β-cell glucose sensitivity, independently of weight loss.

Neil Ruderman described the regulation of adenosine monophosphate kinase (AMPK), a fuel-sensing enzyme that is activated by decreases in a cell's energy state as reflected by an increased ratio of adenosine monophosphate (AMP) to adenosine triphosphate (ATP). During states such as glucose deprivation and exercise, AMPK activation has been shown to restore ATP levels by activating processes that generate ATP (e.g. fatty acid oxidation), and inhibiting others that consume ATP but are not acutely necessary for survival (e.g. fatty acid, TG and protein synthesis). Recent studies suggest that various hormones can also activate or inhibit AMPK. One of these hormones is adiponectin, an adipokine released by the fat cell. Adiponectin has been shown to activate AMPK in liver, muscle, and cultured adipocytes and endothelium. Low plasma levels of adiponectin are associated with obesity, insulin resistance and ectopic lipid deposition in multiple tissues. In humans, they are also associated with a predisposition to both type 2 diabetes and atherosclerotic heart disease. The extent to which these effects of a lack of adiponectin are due to decreased tissue AMPK activity remains to be determined.

Whereas the adipocyte releases upward of 20 hormones, to date the only hormone found to originate in muscle is interleukin-6 (IL-6). It is synthesized and released by muscle in large amounts during sustained exercise, during which its concentration in plasma may increase by 50–100-fold. It has been suggested that this IL-6 acts on the fat cell to increase lipolysis when the exercising muscle needs additional fatty acids as a fuel. IL-6 activates AMPK in both adipose tissue and muscle.

Katherine Cianflone described the regulation of fatty acid transport and storage, especially the influence of acylation-stimulating protein (ASP). People spend a large proportion of their waking hours in the postprandial state. Postprandial lipaemia (increases in blood lipids following a meal) occurs several times daily, resulting in very efficient absorption of dietary fat and redistribution to various tissues. If the dietary intake of fat exceeds the actual needs, the removal process of dietary TGs becomes overloaded, resulting in excessive postprandial lipaemia and accumulation of chylomicrons and remnant particles.

TG clearance occurs as a two-step process: first, the lipoproteins are hydrolysed by lipoprotein lipase (LPL), releasing NEFAs; secondly, NEFAs are taken up into the cell and re-esterified to a storage TG molecule. Numerous studies *in vitro* and *in vivo* have demonstrated that excess generation of NEFAs by LPL, without prompt and rapid clearance into cells, will result in product inhibition of LPL. Therefore, processes that amplify the ability of the adipocyte rapidly to take up and store NEFA as TGs will indirectly increase the efficiency of LPL. ASP is one such hormone that leads to increased glucose transport and TG storage. While ASP has no direct effect on LPL activity, the TG clearance is enhanced through increasing TG storage and relieving NEFA inhibition of LPL. Thus, adipose tissue trapping of LPL-derived NEFAs determines overall LPL activity, which in turn determines the efficiency of TG clearance. In contrast to these effects of ASP on adipose tissue, ASP decreased *in situ* muscle LPL activity, similarly to the effects of insulin.

In human studies, fasting ASP is influenced by diet, body size (obesity), exercise and metabolic status (presence of cardiovascular disease and/or diabetes). At the level of peripheral fatty acid, especially in adipose tissue, the central component

is LPL. But LPL does not act alone; it is supported by a panoply of stimulatory and inhibitory cofactors including ASP. Not only do all these factors contribute to postprandial clearance, but also many of these factors are influenced by dietary factors, creating a complex web of interdependence.

Gabriele Riccardi developed further on postprandial lipid metabolism. Abnormalities are more frequent in individuals with type 2 diabetes and other states of insulin resistance. Since these abnormalities are associated with an increased risk of coronary heart disease, they could be a major factor accounting for the higher rate of cardiovascular diseases observed in these conditions.

The direct role of insulin resistance in the development of postprandial dyslipidaemia has been demonstrated in acute studies, where the confounding effects of hyperglycaemia and hyperinsulinaemia were avoided by using the hyperinsulinaemic glycaemic clamp technique. There is consistent evidence that a high fat intake is associated with both insulin resistance and postprandial lipid abnormalities. Clinical and experimental studies have demonstrated that increasing fat consumption worsens postprandial hypertriglyceridaemia in individuals with type 2 diabetes and/or hyperlipidaemia.

In the KANWU study a diet rich in monounsaturated fatty acids (MUFAs), compared with a diet rich in saturated fatty acids (SFAs), was able to improve insulin sensitivity in non-diabetic individuals. Data on the effects of dietary fatty acid modification on postprandial lipid metabolism in humans, however, are scant and mainly concerned with the acute effects of test meals differing in fatty acid composition. These studies have produced controversial findings, providing some evidence that PUFAs, particularly n-3, induce an attenuated postprandial lipaemic response compared with SFAs and MUFAs.

In a recent study from Riccardi's group a MUFA-rich diet did not modify insulin sensitivity compared with an SFA-rich diet, but induced a higher chylomicron early peak and a significantly lower postprandial response of small VLDL lipoproteins after a standard test meal (rich in saturated fat), compared with the SFA diet. These changes were consistent with increased LPL activity observed in the subcutaneous abdominal adipose tissue both while fasting and postprandially during the MUFA diet.

Challenges for the future

Four different presentations on emerging issues were collected in this session.

Hannele Yki-Järvinen described the fatty liver as a key to insulin resistance and cardiovascular disease. Obesity is not necessary for insulin resistance in humans since severe insulin resistance also characterizes patients lacking subcutaneous fat, such as those with highly active antiretroviral therapy (HAART)-associated lipodystrophy. Both obese and lipodystrophic patients have an increase in the amount of fat hidden in the liver. Liver fat content is closely correlated with fasting insulin concentrations and direct measures of hepatic insulin sensitivity. The amounts of intra-abdominal fat and liver fat are significantly interrelated, but liver fat can be regulated by mechanisms other than those related to the amount of intra-abdominal fat. It is also still controversial whether intra-abdominal fat indeed causes fat accumulation in the liver or is an innocent bystander.

An increase in liver fat content has been shown to predict, independently of obesity and abdominal obesity, type 2 diabetes and cardiovascular disease. This is easily explained by the fact that the liver, once fatty, overproduces most of the known cardiovascular risk factors such as VLDL, glucose, C-reactive protein (CRP), plasminogen activator inhibitor-1 (PAI-1), fibrinogen and coagulation factors.

The causes of interindividual variation in liver fat content independent of obesity are largely unknown, but could involve differences in signals from adipose tissue, such as in the amount of adiponectin produced, and differences in fat intake. Yki-Järvinen's group recently demonstrated that adipose tissue is inflamed in those with a high compared with a low liver fat content, independently of obesity. Adiponectin deficiency characterizes both lipodystrophic and obese insulin-resistant individuals, and serum levels correlate with liver fat content.

Liver fat content can be decreased by weight loss, insulin therapy and a low-fat diet. In addition, treatment of both lipodystrophic and type 2 diabetic patients with PPAR-γ agonists, but not metformin, decreases liver fat and markedly increases adiponectin levels. The fatty liver may help to explain why some but not all obese individuals are insulin resistant and why even lean individuals may be

insulin resistant, and thereby at risk of developing type 2 diabetes and cardiovascular disease.

William Stillwell described the role of polyunsaturated lipids in membrane raft function. One research direction has investigated events that follow the uptake of DHA into animal cell plasma membrane phospholipids. DHA's dynamic shape, consisting of multiple configurations, is very different from what its static, stick structure would indicate. As a result, DHA-containing phospholipids have a wide hydrophobic base compared with their hydrophilic head, and so induce negative curvature strain that severely impacts on the activity of a variety of important membrane proteins. The unusual structure means that DHA-rich membranes are also surprisingly thin and support high permeability, compression, fusion and flip-flop rates.

DHA does not exist in an environment that is independent from other membrane lipids. The interaction of DHA-containing phospholipids with the major lipid raft components cholesterol and sphingomyelin is of particular interest. From a wide variety of biophysical studies, primarily conducted on model lipid monolayers and bilayers, Stillwell proposed a new hypothesis suggesting that DHA may alter plasma-membrane lipid raft structure and hence essential cell signalling events.

Rachel Fisher reported recent studies on fatty acid metabolism and the metabolic syndrome. The characteristic dyslipidaemia associated with the metabolic syndrome, namely high plasma triacylglycerol and low high-density lipoprotein (HDL)-cholesterol concentrations, and a predominance of small, dense low-density lipoprotein (LDL) particles, promotes the development of atherosclerotic disease. However, increased oxidative stress, a proinflammatory state and impaired function of the endothelium have also been proposed as features of the metabolic syndrome, contributing to the progression of atherosclerosis.

In a cross-sectional study of Swedish men with a range of insulin sensitivities, patterns of fatty acid composition representing increased Δ9 desaturase (stearoyl CoA desaturase) activity were associated with the metabolic syndrome. Small, dense LDL particles were not related to the total amounts of SFAs, MUFAs or PUFAs, but fatty acids typically found in milk products were associated with a more favourable LDL profile. Markers of endothelial dysfunction and inflammation were increased in subjects with the metabolic syndrome. Endothelial activity was positively related to urinary biomarkers of oxidative stress, inflammatory markers, total energy intake and adipose tissue content of MUFAs, and negatively to adipose tissue PUFA content.

These results show that the metabolic syndrome is associated with distinct fatty acid patterns. Since the physiological effect of individual fatty acids may not be representative of a whole class of fatty acids, a greater knowledge of the role of individual fatty acids in the aetiology of the metabolic syndrome is important for understanding the associated development of cardiovascular disease.

Fredrik Bäckhed provided the final plenary presentation on intestinal microbiota and metabolism in relation to obesity. The basis of this novel area of research is the fact that the adult human intestine is home to an almost inconceivable number of microorganisms. The size of the population – up to 100 trillion – far exceeds that of all other microbial communities associated with the body's surfaces, and is 10 times greater than the total number of somatic and germ cells. The gut microbiota can be pictured as a microbial organ placed within a host organ: it is composed of different cell lineages with a capacity to communicate with one another and the host. The hypothesis is that differences in gut microbial ecology between individuals may affect the efficiency with which they harvest calories from the diet and store the extracted energy in adipocytes. In this conceptualization, the gut microbiota is viewed as an environmental factor that affects predisposition to obesity versus leanness. This hypothesis has been tested using gnotobiotic mice.

"Conventionalization" of adult germ-free (GF) C57Bl/6 mice with a normal microbiota harvested from the distal intestine (caecum) of conventionally raised mice increased the body fat content by almost 60%, despite reduced food intake. Energy storage is achieved, at least in part, through the following mechanism. Microbial colonization increases energy absorption, which stimulates hepatic lipogenesis through two basic helix-loop-helix/leucine zipper transcription factors: ChREBP and SREBP-1c. These *de novo* synthesized TGs are stored in adipocytes via an LPL-mediated process. The gut microbiota suppresses intestinal epithelial expression of a circulating LPL inhibitor, fasting-induced adipose factor (Fiaf, also known as angiopoietin-like protein-4). Comparisons of GF and conventionalized (CONV-D) WT and *Fiaf*$^{-/-}$

mice established Fiaf as a physiologically important regulator of LPL activity *in vivo*, and a key modulator of the microbiota-induced increase in fat storage. These results suggest that the gut microbiota is an important environmental factor that affects energy harvest from the diet and energy storage in the host.

Diet-induced obesity (DIO) can be induced in rodents by feeding them a high-caloric "Western diet", which is rich in dietary fat (20% vs 5% in chow diet). CONV-D mice became obese after 8 weeks on this diet, while the GF mice were resistant to DIO. Obese animals had a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes. These results indicate that obesity affects the population structure of the gut microbiota, and suggest that intentional manipulation of this community may be useful for regulating energy balance in obese individuals.

Recommendations and practical implications for product development

This final session of the symposium will be reviewed in a forthcoming issue of the Scandinavian Journal of Food and Nutrition.

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