FINAL REPORT

Metabolism and toxicity of dietary trans-fatty acids

Research Project K 125201

Method developments

Health effects of dietary trans fatty acids (TFAs) receive a growing attention; while very little is known about the metabolism of these special food components. In vitro studies carried out in cultured cells provide an efficient and standardizable approach to follow the metabolic fate of TFAs, but it requires suitable techniques for the quantitative measurement of FAs and derivatives in cell samples.

Quantitative analysis of diglycerides, triglycerides and ceramides in cell cultures by using HPLC-MS/MS

A sensitive, reproducible reverse-phased high performance liquid chromatography electrospray tandem mass spectrometry (HPLC-ESI-MS/MS) method with simple sample preparation was developed for the simultaneous determination of a wide range of ceramides and diglycerides (DGs) in cultured cells. Chromatographic separation of the compounds was achieved in a 14-minute run using a C8 column with a gradient elution by methanol and 10 mM ammonium acetate buffer as mobile phase at a flow rate of 0.5 ml/min. Various ceramides and DGs were detected with a triple quadrupol system in multiple reaction monitoring mode, which is based on a soft positive electrospray ionization. The usual sample preparation process was shortened by the application of pure methanol for the extraction instead of the widely used methanol/chloroform mixture. C17:0 ceramide which does not occur in the cell samples, was used as an internal standard. The sample preparation process was optimized and the methodology was tested on HepG2 human hepatocarcinoma cells. Our results clearly showed accumulation of some ceramides and DGs in the cells treated with BSA-conjugated palmitate for 8 hours. Since both ceramides and DGs are important lipid intermediates and signal messengers, alteration in their cellular levels have major impact on cell functions, and thus our novel analytic method can be widely used in lipotoxicity research. The presented technique has been further developed to measure various triglycerides (TGs) as well. [Per Pol Chem Eng. 2020;64(4):421-429. doi: 10.3311/PPch.15357]

Development of a gas chromatography – flame ionization detection (GC-FID) method to analyze the FA profile of cultured cells or tissues, and its application to study cellular incorporation of TFAs in RINm5F rat insulinoma cells and HepG2 human hepatocellular carcinoma cells

We developed and validated a simple and reliable method for the sensitive and accurate quantification of a large group of medically relevant FAs – both esterified or amidified and free FAs – in cell samples by using GC-FID. Sample preparation uses a fast one-step and chloroform-free process for simultaneous extraction and esterification, and chromatographic separation is achieved in 25 min using a Zebron ZB-88 capillary column. A linear calibration (of R2 >0.99) was obtained in the concentration range of 1-200 µg/mL for each FA. Recovery rate was 82% for samples of non-esterified FAs and >95% for complex lipids, such as ceramides, diglycerides and triglycerides. The LOD and LOQ were below 0.5 µg/mL, and a robust method precision was achieved (RSD% was below 6% for each lipid classes). This method was first tested on cultured RINm5F cells with or without FA treatment at close to physiological concentration, and then also successfully applied on HepG2 treated with various BSA-conjugated fatty acids (palmitate, oleate, elaidate, vaccinate and combinations). [Per Pol Chem Eng. 2021;65(2):149-157. doi: 10.3311/PPch.16646]

Metabolism, cellular toxicity and protective effect of dietary TFAs in insulinoma and hepatoma cells

Toxicity of TFAs and its correlation with ceramide and DG accumulation in insulinoma cells

High FA levels are deleterious to pancreatic β-cells, largely due to the accumulation of biosynthetic lipid intermediates, such as ceramides and DGs, which induce ER stress and apoptosis. Toxicity of palmitate (16:0) and oleate (18:1 cis-Δ9) has been widely investigated, while very little data is available on the cell damages caused by elaidate (18:1 trans-Δ9) and vaccenate (18:1 trans-Δ11), although the potential health effects of these TFAs received great publicity. We compared the effects of these four FAs on cell viability, apoptosis, ER stress, JNK phosphorylation and autophagy as well as on ceramide and DG contents in RINm5F insulinoma cells. The protein markers of ER stress (e.g. PDI, BiP, IRE1, PERK, ATF6, phosphorylated eIF2alpha, IRE1, PERK) and those of apoptosis (cleaved Caspase-3 and induction of CHOP) were assessed by Western blotting, the ER stress-induced editing of XBP-1 mRNA was detected by RT PCR and restriction endonuclease cleavage, intensity of autophagy was examined by both detecting the protein marker (i.e. the ratio of LC3-II to LC3-I) by visualizing autophagy-specific subcellular morphological changes using transmission electron microscopy in the FA treated RINm5F cells. Changes in the FA profile were quantitated through GC-FID analysis of extracted and derivatized FAs specimens, while the intracellular levels of selected ceramides and DGs were measured by using LC-MS/MS analysis. Similarly to oleate and unlike palmitate, TFAs reduced cell viability only at higher concentration, and they had mild effects on ER stress, apoptosis and autophagy. Palmitate increased ceramide and DG levels far more than any of the unsaturated fatty acids; however, incorporation of TFAs in ceramides and DGs was strikingly more pronounced than that of oleate. This indicates a correlation between the accumulation of lipid intermediates and the severity of cell damage. Our findings reveal important metabolic characteristics of TFAs that might underlie a long term toxicity and hence deserve further investigation. [Food Chem Toxicol. 2019;124:324-335. doi: 10.1016/j.fct.2018.12.022]

Effect of cis- and trans-monounsaturated FAs on palmitate-induced toxicity and ceramide and DG accumulation in insulinoma cells

As it was shown earlier, both TFAs caused only a weak stress and a mild damage in RINm5F insulinoma cells compared to palmitate. However, incorporation of TFAs in ceramides and DGs was strikingly more pronounced than that of oleate. This has been continued by addressing the possible protective action of TFAs on palmitate-treated cells with special regards to apoptosis, ER stress and the underlying ceramide and DG accumulation. Both TFAs significantly improved cell viability and reduced apoptosis in palmitate-treated cells. They mildly attenuated palmitate-induced XBP-1 mRNA cleavage and phosphorylation of eukaryotic initiation factor 2α (eIF2α) and stress-activated protein kinase (SAPK)/c-Jun N-terminal kinase (JNK), but they were markedly less potent than oleate. Accordingly, all the three unsaturated FAs markedly reduced cellular palmitate incorporation and prevented harmful ceramide and DG accumulation. These findings further support the proposed correlation between the accumulation of lipid intermediates and the severity of the damage in insulinoma cells. However, it is noteworthy that more elaidate or vaccenate than oleate was inserted into ceramides and DGs in our experiments. Most importantly, our results show the remarkable protective effect of trans FAs against palmitate-induced short-term toxicity, particularly ER stress and apoptosis in insulinoma cells on the one hand, yet a marked and important difference between the utilization of trans FAs and oleate in ceramide and DG synthesis, with probable long-term health impacts on the other hand. The molecular background behind the preferred incorporation of trans- vs. cis-unsaturated FAs into ceramides as well as the potential inhibition of palmitate uptake by simultaneously administered unsaturated FAs remain to be elucidated. [Int J Mol Sci. 2020;21(7):2626. doi: 10.3390/ijms21072626]

Different metabolism and toxicity of TFAs compared to cis-oleate in HepG2 cells

TFAs are not synthesized in the human body but are generally ingested in substantial amounts. The widespread view that TFAs, particularly those of industrial origin, are unhealthy and contribute to obesity, cardiovascular diseases and diabetes is based mostly on in vivo studies, and the underlying molecular mechanisms remain to be elucidated. Here, we used a hepatoma model of palmitate-induced lipotoxicity to compare the metabolism and effects of the representative industrial and ruminant TFAs, elaidate and vaccenate, respectively, with those of cis-oleate. Cellular FAs, TGs, DGs and ceramides were quantitated using chromatography, markers of stress and apoptosis were assessed at mRNA and protein levels, ultrastructural changes were examined by electron microscopy and viability was evaluated by MTT assay. While TFAs were just slightly more damaging than oleate when applied alone, they were remarkably less protective against palmitate toxicity in cotreatments. These differences correlated with their diverse incorporation into the accumulating DGs and ceramides. Our results provide in vitro evidence for the unfavorable metabolic features and potent stress-inducing character of TFAs in comparison with oleate. These findings strengthen the reasoning against dietary trans fat intake, and they can also help us better understand the molecular mechanisms of lipotoxicity. [Int J Mol Sci. 2022;23(13):7298. doi: 10.3390/ijms23137298]

Cellular protection against lipotoxicity – FA desaturation and adaptive ER stress

Investigation of the putative rate-limiting role of electron transfer in fatty acid desaturation

The stress caused by permanently elevated FA levels can lead to cellular dysfunction or even cell death, which contributes to the development of pathological conditions, such as cardiovascular diseases, non-alcoholic fatty liver disease and type 2 diabetes. The most severe lipotoxicity is caused by an unbalanced oversupply of saturated FAs (e.g. palmitate), while substantially milder damages are caused by unsaturated FAs of either cis or trans configuration, and hence the efficiency of stearoyl-CoA desaturase (Scd1) is an important factor of resistance. A novel oxidoreductase has been shown to protect cells against palmitate toxicity, so we aimed to test whether Scd1 itself or the associated electron supply is rate-limiting for cellular desaturase activity. The FA profile was assessed by GC-FID analysis in transiently transfected HEK293T cells. Overexpression of Scd1 resulted in a marked elevation of unsaturated/saturated FA ratio, but this effect was not achieved by overexpression of the Scd1-related electron transfer proteins. The electron supply did not become rate-limiting even in palmitate-treated cells or in cells of enhanced Scd1 expression and activity. In accordance with other observations, our findings indicate that Scd1 enzyme itself catalyzes the rate-limiting step of FA desaturation, and this function cannot be facilitated by reinforcing the electron supply of the enzyme in this cell line. [FEBS Lett. 2020;594(3):530-539. doi: 10.1002/1873-3468.13622]

Molecular mechanisms underlying the elevated expression of a potentially type 2 diabetes mellitus associated Scd1 variant

Disturbances in lipid metabolism related to excessive food intake and sedentary lifestyle are among major risk of various metabolic disorders. Stearoyl-CoA desaturase-1 (Scd1) has an essential role in these diseases, as it catalyzes the synthesis of unsaturated fatty acids, both supplying for fat storage and contributing to cellular defense against saturated fatty acid toxicity. Recent studies show that increased activity or overexpression of Scd1 is one of the contributing factors for type 2 diabetes mellitus (T2DM). We aimed to investigate the impact of the common missense rs2234970 (M224L) polymorphism on Scd1 function in transfected cells. We found a higher expression of the minor Leu224 variant, which can be attributed to a combination of mRNA and protein stabilization. The latter was further enhanced by various fatty acids. The increased level of Leu224 variant resulted in an elevated unsaturated: saturated fatty acid ratio, due to higher oleate and palmitoleate contents. Accumulation

of Leu224 variant was found in a T2DM patient group, however, the difference was statistically not significant. In conclusion, the minor variant of rs2234970 polymorphism might contribute to the development of obesity-related metabolic disorders, including T2DM, through an increased intracellular level of Scd1. [Pending editor decision on the revised manuscript at Genes, ISSN: 2073-4425]

Pharmacological protection of the cells in ER stress by promoting autophagy-dependent survival
ER stress and autophagy are simultaneously induced in lipotoxicity. The former might trigger apoptotic cell death, while the latter is fundamentally protective and favours cell survival. It is, therefore, intriguing whether certain pharmacological interventions can promote the autophagic protection, and by this means alleviate the lipotoxic cell injury. The life-and-death decision in ER stress response is defined by a crosstalk between autophagy, apoptosis and mTOR-AMPK pathways, where the transient switch from autophagy-dependent survival to apoptotic cell death is controlled by GADD34. We investigated whether epigallocatechin-3-gallate (EGCG), the major polyphenol of green tea, can promote autophagy-dependent survival in ER stress. Our findings revealed that EGCG treatment is able to extend cell viability and it induces cytoprotective autophagy by down-regulating mTOR and up-regulating AMPK pathways. We confirmed that EGCG-induced autophagy is mTOR-dependent and PKA-independent; furthermore, it also required ULK1. We show that pre-treatment of the cells with EGCG diminishes the negative effect of GADD34 inhibition (by guanabenz or siGADD34 treatment) on autophagy. EGCG was able to delay apoptotic cell death by up-regulating autophagy-dependent survival even in the absence of GADD34. These data suggest a novel role for EGCG in promoting cell survival via shifting the balance of mTOR-AMPK pathways in ER stress, and they also offer the possibility of diminishing lipotoxic cell damage by EGCG treatment. [Oxid Med Cell Longev. 2018;2018:6721530. doi: 10.1155/2018/6721530]

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