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REVIEW

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Mechanisms of lipotoxicity-induced dysfunction and death of human pancreatic beta cells under obesity and type 2 diabetes conditions

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Summary

The term "pancreatic beta-cell lipotoxicity" refers to the detrimental effects of free fatty acids (FFAs) on a wide variety of cellular functions. Basic research in the field has primarily analyzed the effects of palmitic acid and oleic acid. The focus on these two physiological FFAs, however, ignores differences in chain length and degree of saturation. In order to gain a comprehensive understanding of the lipotoxic mechanisms, a wide range of structurally related FFAs should be investigated. Structure–activity relationship analyses of FFAs in the human EndoC-βH1 beta-cell line have provided a deep insight into the mechanisms of beta-cell lipotoxicity. This review focuses on the effects of a wide range of FFAs with crucial structural determinants for the development of lipotoxicity in human beta cells and documents an association between increased triglyceride stores in obesity and in type 2 diabetes.

KEYWORDS
free fatty acids, human EndoC-βH1 beta-cell line, lipotoxicity, obesity, type 2 diabetes mellitus

1 | INTRODUCTION

The availability of glucose and lipids in abundance, which began in the Western World in the late 1950s and early 1960s and is meanwhile widely prevalent in many areas of the world, goes along with overweight and obesity. It is also the cause of an ever increasing incidence of type 2 diabetes mellitus (T2DM).[1,2] This is a problem resulting to a large extent from an uneven nutrient availability in different parts of the world. It could be solved in an ideal world, in which, however, we are not living on this planet. Thus, medicine has to struggle with the devastating health problems and the socioeconomic consequences of obesity and T2DM. That is why it is all the more important to elucidate the underlying causes of lipotoxicity. Increased concentrations of free fatty acids (FFAs) in the circulation accompany the development of obesity and T2DM.[3] With their toxic potential, the FFAs hit the pancreatic beta cells, a cell type particularly sensitive to oxidative stress due to the low antioxidative defense equipment,[4–7] which make this cell type so vulnerable.[8]

A focus upon single popular physiological fatty acids such as palmitic acid and oleic acid in many scientific studies has not yet deciphered the whole spectrum of mechanisms of FFA toxicity, as documented in recent review articles of this research field.[9–11] Only the use of a wide range of FFAs of different chemical structure has opened the perspective for a full understanding of lipotoxicity.[12–14]

The availability of the well-differentiated human EndoC-βH1 beta-cell line,[15] which has been characterized in many studies both with respect to its physiological[16] and pathophysiological[17] features,

Abbreviations: ATP, adenosine triphosphate; FFA, free fatty acid; H_2O_2, hydrogen peroxide; sFFA, saturated fatty acid; T2DM, type 2 diabetes mellitus; uFFA, unsaturated fatty acid.

shows an overall great comparability to the situation in primary human beta cells. Meanwhile, it is therefore also a well-established novel biological tool for studies on the toxicity of saturated and unsaturated FFAs,[13,14] which are oxidized in the mitochondrial and peroxisomal β-oxidation.[18,19] It allowed the elucidation of the mechanisms of FFA toxicity in pancreatic beta cells.[6,20] This comprises also the relevance for the situation in primary human beta cells, as documented in a study comparing human EndoC-βH1 beta cells with beta cells in primary human islets.[13]

2 | CHAIN LENGTH-DEPENDENT TOXICITY OF FFAS IN HUMAN BETA CELLS

In human EndoC-βH1 beta cells, both saturated FFAs (sFFAs) and unsaturated FFAs (uFFAs) are toxic.[13,14] A comparison of a number of different cytotoxicity tests (annexin V staining, PI staining, and caspase-3 activity) provided comparable results,[13] with the caspase-3-activity methodology being the most sensitive.[13] Therefore, this latter method has been widely adopted for extensive FFA toxicity analyses.[14,21,22] Beta-cell toxicity increases continuously with chain length, as documented in a study at a standardized concentration of 500 µmol/L for all tested FFAs[14] (Figure 1A). While short- and medium-chain FFAs with a chain length of up to C12 exhibit no beta-cell toxicity, long-chain FFAs (C16-C18) are toxic as documented by an increased caspase-3 activity.[14]

Myristic acid (C14:0) in the transition zone between medium- and long-chain fatty acids is still devoid of beta-cell toxicity[14] but starts to develop some toxicity when the expression of the enzyme stearoyl-CoA desaturase 1 (SCD1) is suppressed.[22] Beta-cell toxicity of the longer FFA stearic acid (C18:0) is greater than that of the shorter FFA palmitic acid (C16:0).[14] The toxicity of the monounsaturated FFAs palmitoleic acid (C16:1) and oleic acid (C18:1) is less pronounced when compared to their saturated counterparts of the same chain length and at the same concentration, with a toxicity of oleic acid being equivalent to that of palmitic acid and therefore more toxic than palmitoleic acid[14] (Figure 1A). While the toxic potential of palmitic acid and stearic acid has been consistently emphasized in the literature, reports on the effects of palmitoleic and oleic acid are conflicting.[12,13,23,24]

The beta-cell toxicity of FFAs increases strongly in the case of the very long-chain FFAs.[14] Already in the transition zone, this much greater toxicity is evident in the case of nonadecanoic acid (C19:0)[14,25] and in the case of nonadecenoic acid (C19:1).[14] The toxicity of the very long-chain FFAs arachidic acid (C20:0) and gondoic acid (C20:1) is even greater.[14]

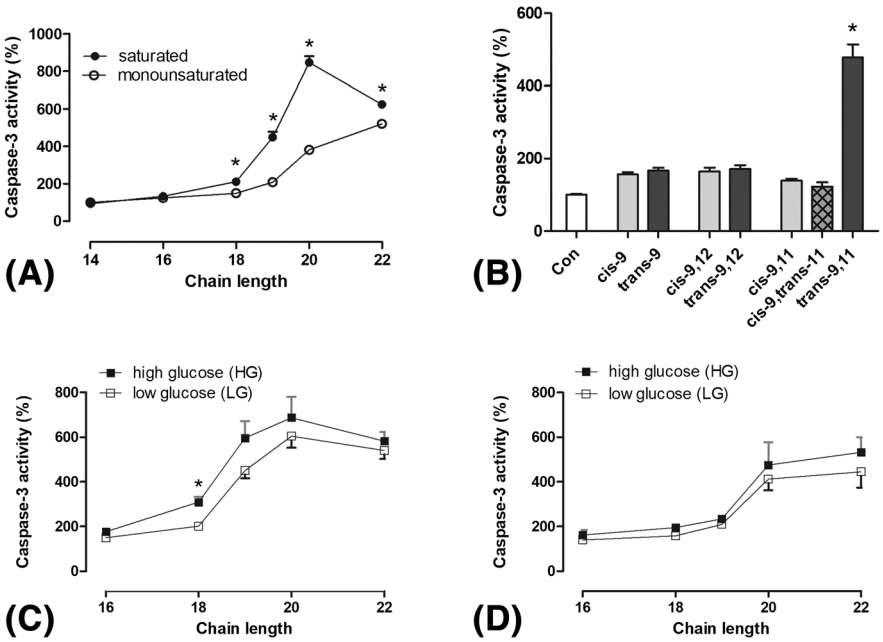


FIGURE 1 Lipotoxicity of various long- and very long-chain saturated or unsaturated FFAs in human EndoC-βH1 beta-cells. Concentration-dependent toxicity (as documented by caspase-3 increase) of saturated FFAs and monounsaturated FFAs in the human EndoC-βH1 beta-cell line (A). Toxicity (as documented by caspase-3 increase) of *cis,trans* unsaturated FFAs in the human EndoC-βH1 beta-cell line (B). Opposing toxic effects (as documented by caspase-3 increase) of saturated (C) and monounsaturated (D) FFAs at a low (5.5 mM) and at a high (25 mM) glucose concentration in the human EndoC-βH1 beta-cell line. All FFAs were dissolved in 90% ethanol solution, and the toxicity was tested after dilution in the presence of a final ethanol concentration of ≤0.9%. The concentrations of all FFAs presented in this figure were 500 µmol/L in the presence of 1% bovine serum albumin in the incubation medium. Control experiments without FFAs were performed at the same final ethanol concentration in the presence of 1% BSA in the incubation medium. All data are adopted from Plötz et al.[14] Data are means ± SEM from four to nine individual experiments. *$p < 0.01$ as compared to the same FFA with one double bond (A), low glucose (C,D) (Student's *t* test), or compared to control (B) (ANOVA, Dunnett's multiple comparison test).

3 | TOXICITY OF THE PHYSIOLOGICALLY RELEVANT LONG-CHAIN FATTY ACIDS IN HUMAN BETA CELLS

The physiological FFAs (C16-C18) in the circulation, both saturated and unsaturated, mostly originate from dietary intake, while FFA synthesis from glucose primarily yields palmitic acid. However, palmitic acid can be converted endogenously through chain length elongation and desaturation into other FFA species. In addition, the total plasma FFA concentration in humans shows wide variations, depending on diet as well as on the nutritional and health status.[26–28] Thus, plasma FFA concentrations depend not only on FFA intake but also on endogenous lipolysis in the triglyceride stores.

The two most prominent physiological FFAs, palmitic acid (PA) (C16:0) (64 nmol/ml human plasma concentration)[29] and oleic acid (OA) (C18:1) (80 nmol/ml human plasma concentration),[29] contribute 4/5th of the physiological FFA concentration in the circulation but comprise only 2/5th of the total toxic potential of the four prominent physiological FFAs when compared at a 500 µmol/L concentration in vitro (Figure 1A).[14] This is due to the larger toxic potential in particular of stearic acid (SA) (C18:0) (Figure 1A)[14] in spite of its lower plasma concentration (22 nmol/ml human plasma concentration)[29] in contrast to the low toxic potential of palmitoleic acid (PO) (C16:1)[14] (Figure 1A) along with its low plasma concentration (15 nmol/ml human plasma concentration).[29] Thus, the two C16 physiological long-chain FFAs contribute 1/4th of the toxic potential, while the two C18 physiological FFAs contribute 3/4th of the toxic potential[14] (Figure 1A). So the classification of oleic acid as a beneficial and of palmitic acid as an unfavorable FFA is a wrong popular myth at least with respect to human pancreatic beta-cell toxicity. On the other hand, it is a blessing for beta-cell health that oleic acid represents the much larger proportion in the circulation relative to stearic acid, because otherwise, the high proportion of C18 FFAs in the circulation would be a disaster for beta-cell wellbeing. Table 1 enumerates the major differences between palmitic, stearic acid and oleic acid.

The mechanism underlying the toxicity of the physiological long-chain FFAs (C16-C18) is an increased production of H_2O_2 in peroxisomal β-oxidation[14,20,30,31] especially in pancreatic beta cells, which are not equipped with an efficient system for removal of H_2O_2 in the peroxisomes.[4–7] This is the cause for the apoptotic beta-cell death by the physiological FFAs as documented by electron microscopy[31] and confirmed by caspase-3 activity measurements.[14,22] In the case of the polyunsaturated FFAs, ferroptosis is a contributing cell death mechanism of significant impact, which only occurs when the high expression level of the antioxidative GPX4 in human beta cells is suppressed.[32] The great toxicity of the non-physiological very long-chain FFAs (>C18) goes along with an additional increase of H_2O_2 production in the mitochondria,[14] which is accompanied by pronounced ATP loss and an increase of cardiolipin peroxidation in the beta cells. Additional mechanisms have been considered in the context of lipotoxicity, such as inflammation, autophagy, and ER stress.[22,33–35] With respect to the toxicity of the whole spectrum of the different FFAs, however, oxidative stress has turned out to be the only predominant mechanism of FFA toxicity in human beta cells.[14]

TABLE 1 Comparison of the toxicity features of the most prevalent FFAs in humans, the sFFAs palmitic acid (PA), stearic acid (SA), and the uFFA oleic acid (OA) (at a 500 µmol/L concentration each) in human EndoC-βH1 beta cells.

The uFFA OA (18:1) is less toxic than the sFFA SA (18:0) because of the double bond in its structure.
The monounsaturated FFA OA (18:1) is slightly more toxic than the sFFA PA (16:0), because it is two C atoms longer though it has a double bond.
The toxicity of the uFFAs is not potentiated by glucose in contrast to that of very long-chain sFFAs (≥18:0).
ER stress starts to occur after treatment of cells with very long-chain sFFAs (≥C18) but is not induced by uFFAs.

4 | EFFECTIVE CONCENTRATIONS OF FFAS WITH RESPECT TO THEIR TOXICITY IN HUMAN BETA CELLS

Free unbound concentrations of the FFAs represent their effective toxic concentrations.[36] However, it is crucial to recognize that the structural properties of the FFAs are also of particular relevance for their toxicity. It is evident that the free unbound concentration is dependent on the chemical structure of the FFA,[37,38] the relation between FFA concentration and bovine serum albumin (BSA) concentration,[36,39–41] and the metabolic status (i.e., fed vs. fasted) and the health status.[42,43] For example, in the presence of 1% albumin and 500 µmol/L FFA, the amount of free unbound FFA has been calculated to be 27 nmol/L for palmitic acid and 47 nmol/L for oleic acid.[36,44] Nevertheless, the toxicity towards human beta cells of these two FFAs is comparably high even though the free unbound concentration of oleic acid is almost twice as high as that of palmitic acid,[13,14] implicating that the free unbound FFA fraction cannot be the only responsible factor for the toxicity of the FFAs. Since the total plasma FFA concentrations in human individuals show wide variations and the effective FFA concentrations reaching the beta cells are hardly known, in vitro experiments with FFAs will never fully reflect the in vivo situation and should therefore be interpreted with caution. Nevertheless, differences in the free unbound FFA concentration can affect the extent of toxicity. This aspect can be an element of the toxic potential not only in the in vitro but also in the in vivo situation, emphasizing that differences in the extent of protein binding can be a physiochemical component of their toxicity.[43] Due to the addition of BSA in virtually all in vitro lipotoxicity studies, the free unbound FFA concentrations are reduced. However, the presence of BSA in the incubation experiments is mandatory in order to dissolve the lipophilic FFAs. On the other hand, this approach adopted in in vitro experiments also reflects the in vivo situation, where lipids bind to proteins consequently reducing the free unbound concentration of the FFAs. Therefore, it is important to consider the BSA concentration when comparing toxicities reported in different studies.

5 | ERUCIC ACID AS A PROTOTYPE OF A VERY LONG-CHAIN FFA WITH SEVERE TOXICITY IN HUMAN BETA CELLS

Contents of highly toxic very long-chain fatty acids (>18 C atoms)[14] in vegetable oils are negligible nowadays, and consequently, concentrations of these very long-chain FFAs are also very low in human plasma.[26,29] This limits the detrimental effects of edible oils on beta cells.[45] However, there is one notable prominent historical exception. This is the situation related to the old rapeseed cultivars with a high content of erucic acid (C22:1) in the range of 1/3rd–2/3rd of the total FFA content.[46] In the early days, old rapeseed oil was used only for lighting in oil lamps but not for nutritional purposes due to its pronounced cardiotoxicity.[47] Present day 00 rapeseed cultivars (also called canola rapeseed cultivars), which contain typically between 0.1% and 0.01% erucic acid,[46] lack this enormous toxicity of old rapeseed oil[45,46] along with minimal mitochondrial damage in the pancreatic beta cell.[14] Therefore, 00 rapeseed oil is nowadays a very popular edible oil. However, there is one lesson to be learned from the toxicity of old rapeseed oil, which is consistently documented in in vitro studies with human beta cells.[45] It proofs that the chain lengths of the very long-chain FFAs make them incompatible with human health and must therefore be excluded from the daily diet. It documents convincingly why the physiological FFAs (C16-C18) have the maximal allowable chain length, which is just still tolerable for inclusion in edible vegetable oils. This conclusion is in agreement with observations made in a study analyzing a range of FFA compositions mimicking edible oils with respect to beta-cell toxicity.[45]

While rapeseed cultivars with even higher contents of erucic acid have been bred for industrial purposes (e.g., for use as lubricants or in biodiesel as well as in cosmetics), plant breeders have been reluctant with respect to the generation of cultivars for healthy nutrition with little beta-cell toxic potential.[48] The ultimate aim must be for this reason to minimize the need for peroxisomal β-oxidation. Rapeseed cultivars with high contents of oleic acid (C18:1) as well as with saturated medium-chain FFAs, that is, myristic acid (C14:0), lauric acid (C12:0), and even shorter FFAs (C8:0 and C10:0), exist.[49–51] Rapeseed cultivars with high contents of myristoleic acid (C14:1) and/or palmitoleic acid (C16:1), which would be even more favorable than corresponding cultivars with high contents of saturated FFAs, would thus be ideally suited to keep beta cells healthy, as documented in in vitro studies analyzing the beta-cell toxicity of various FFA compositions.[14,45] However, such cultivars do not exist, apparently due to a lack of availability of specific desaturases required for expression in rapeseed cultivars.

6 | IMPLICATIONS FOR THE COMPOSITION OF A BETA-CELL PROTECTIVE DIET WITH RESPECT TO ITS LIPID COMPONENT

An analysis of the beta-cell toxicity of a number of different compositions (four or more) of FFAs mimicking the composition of edible oils allowed the conclusion that protection against saturated FFA toxicity was increased in the presence of a high content of short- and medium-chain FFAs but interestingly not in the presence of unsaturated FFAs.[45] As this is an in vitro study on the toxicity of various lipid compositions, it is limited with respect to a transfer to the in vivo situation. Nonetheless, these findings may provide new insights with respect to the FFA compositions that should be selected for cooking to protect pancreatic beta cells against lipotoxicity. For frying purposes solid fats (e.g., coconut or palm kernel) with their high content of beta-cell healthy medium-chain and predominantly saturated FFAs are a good option, to minimize the generation of *trans* FFAs. Selecting a universally suitable oil for use in the kitchen (e.g., for salad dressings and cooking) is a challenge. The oil has to be liquid as well as thermally stable, and it should be without significant beta-cell toxicity. None of the commercially available plant oils really fulfill this triple purpose optimally, as documented in a study that mimicked edible oils in terms of beta-cell toxicity.[45] In order to translate the results of this study to the in vivo situation, a good solution could be a self-production of a mixture of plant oil (i.e., olive, sunflower, and rapeseed) supplemented with a plant fat (e.g., coconut and palm kernel) containing a decent content of short- and medium-chain FFAs. Such a composition represents a beta-cell healthy mixture that is liquid and thermally stable. On the other hand, studies in human EndoC-βH1 beta cells revealed that a larger component of long-chain monounsaturated FFAs instead of short- and medium-chain FFAs in such a mixture is not a sensible alternative since an addition of monounsaturated FFAs to such a mixture does not dampen toxicity.[13,45] In any case, these recommendations are more complex than the view traditionally held in the cardiovascular research field favoring a lipid composition with a dominant monounsaturated FFA component, in particular of oleic acid,[52,53] as it is typically present in olive oil. Notwithstanding these above considerations, a restriction of lipid intake in the daily diet may be ultimately a very favorable solution.

7 | GREATER TOXICITY OF *TRANS* UFFAS IN HUMAN BETA CELLS

Trans uFFAs, fatty acids with double bonds in *trans* configuration, are consistently somewhat more toxic than uFFAs in *cis* configuration, when compared at a 500 μmol/L concentration (Figure 1B).[14] Some representatives of uFFAs in *trans* configuration are even significantly more toxic, especially those *trans* uFFAs with two or more consecutive odd-numbered double bonds. But this is not the case with uFFAs containing even-odd-numbered alternating double bonds (Figure 1B). Interestingly, replacement of only a single double bond in trans-9,11 octadecadienoic acid (ODDA) by a double bond in *cis* configuration (cis-9,trans-11 ODDA) is sufficient to abolish the markedly increased toxicity caused by the second odd-numbered double bond in this *trans* uFFA[14] (Figure 1B). The significant content of *trans* uFFAs in butter is therefore a relative disadvantage of this fat of bovine origin in view of the results obtained in in vitro studies with various lipid mixtures in

human beta cells, while the relatively high content of (healthy) medium-chain FFAs in butter is advantageous.[45] Fortunately, concerns regarding high contents of *trans* uFFAs in margarine have largely been addressed through modern production technologies in the food industry. As a result, the content of *trans* fats in modern margarine is considerably lower alleviating the major health concerns associated with high *trans* uFFA consumption.

8 | STEPS IN FFA METABOLISM RESPONSIBLE FOR REDUCED TOXICITY OF *CIS* UFFAS AND FOR INCREASED TOXICITY OF *TRANS* UFFAS IN HUMAN BETA CELLS

In the case of an uFFA with odd-numbered double bonds, a conversion to a *cis*-Δ^3-enoyl-CoA occurs after several cycles of β-oxidation.[19] It is compulsory to transform this degraded uFFA to *trans*-Δ^2-enoyl-CoA through enoyl-CoA isomerase,[19] which appears to retard the rate of chain shortening in the β-oxidation towards generation of an acetyl-CoA molecule and a chain shortening by two C atoms of the acyl-CoA molecule (Scheme 1). This procedure takes place in the mitochondrial β-oxidation along with the generation of NADH and $FADH_2$, except when the FFAs are very long (>C18). Then, the initial step of β-oxidation takes place under generation of H_2O_2 in the peroxisome (Scheme 1).[19,54] Since the peroxisomal metabolism is incomplete, the acyl-CoAs are thereafter transferred for further processing to the mitochondrial β-oxidation (Scheme 1).[54] The retardation of the chain shortening process in the case of uFFAs reduces the generation of toxic H_2O_2 by slowing down metabolic flux through the β-oxidation pathway,[14] providing an explanation for the lesser toxicity of each uFFA when compared with its sFFA counterpart of the same chain length and at the same concentration (Figure 1A).

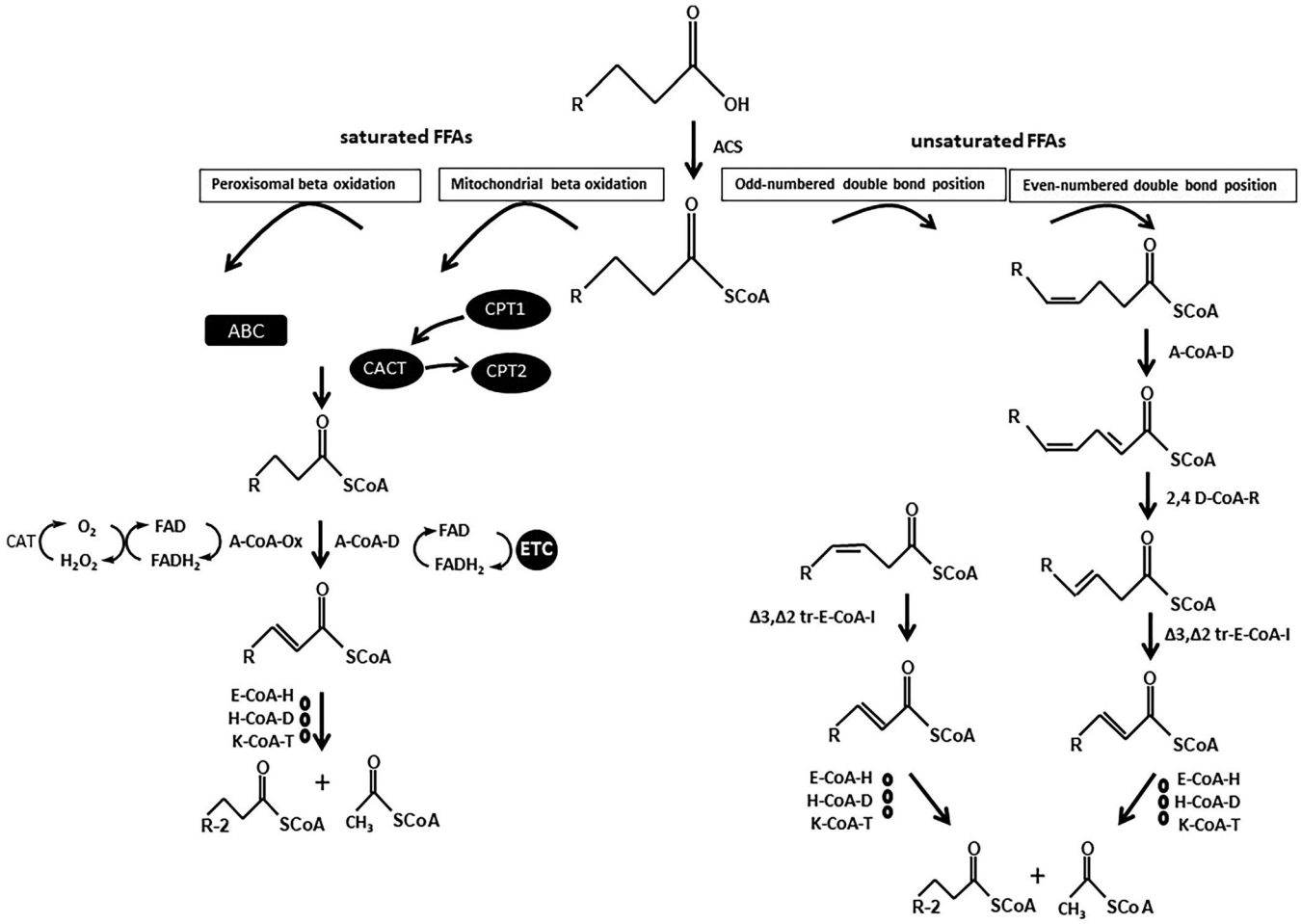
Opposite is the situation in the case of the *trans* uFFAs, which are more toxic than their *cis* counterparts, because the metabolic flux rate through the β-oxidation pathway is apparently accelerated with a greater generation of toxic H_2O_2.[14] This is since a conversion to the *trans* double bond is not required as it is the case for uFFAs with *cis* double bonds (Figure 1B).[19] The metabolism of *trans*-configured uFFAs is less dependent upon the complex enzymatic β-oxidation machinery than that of the *cis*-configured uFFAs.[19]

Studies with various octadecenoic acids (ODAs) and octadecadienoic acids (ODDAs) have clearly documented that the rate of availability of the final FFA product in *trans*-Δ^2 configuration is reduced in the β-oxidation through the double bonds before the respective enoyl-CoA enters the final steps of acetyl-CoA generation.[14] So the lesser toxicity of *cis* uFFAs and the greater toxicity of *trans* uFFAs (Figure 1B) of identical chain length can be led back to the same peroxisomal phenomenon, namely, to the lesser and the greater production of beta-cell toxic H_2O_2, respectively.[14] This conclusion is supported by the observation that inhibition of the stearoyl-CoA desaturase 1 (SCD1), which catalyzes the transformation of sFFAs into *cis*-configured uFFAs by small chemical molecules or through inhibition of gene expression, increases the toxicity of sFFAs.[21,22]

On the other hand, it must be clearly stated that a proof of mitochondrial toxicity in beta cells by very long-chain FFAs is vice versa also a proof that the lower toxicity of physiological long-chain FFAs is due to a lack of mitochondrial toxicity. If mitochondrial toxicity would be a relevant component in the toxicity of the physiological long-chain FFAs, it would also be detectable after exposure to these FFAs, which, however, is not the case.[14]

9 | DEFECTS IN FFA METABOLISM OF DEDIFFERENTIATED INSULIN-PRODUCING BETA-CELL LINES ARE RESPONSIBLE FOR LACK OF TOXICITY OF UFFAS

Pancreatic beta cells like virtually all other mammalian cell types are endowed with a mitochondrial and a peroxisomal β-oxidation pathway for oxidative degradation of FFAs.[18,19,55] While reducing equivalents are generated in mitochondrial β-oxidation, toxic hydrogen peroxide (H_2O_2) is generated in the peroxisomal β-oxidation (Scheme 1). Pancreatic beta cells are badly protected against H_2O_2 toxicity.[4–7] Medium-chain FFAs are typically degraded in the mitochondrial β-oxidation, whereas long-chain and very long-chain FFAs are initially chain-shortened in the peroxisomal β-oxidation before they are transferred for further degradation into the mitochondria.[54] This is true both for sFFAs and uFFAs.[18,19] However, in poorly differentiated insulin-producing cell lines, the capability for degradation of uFFAs such as oleic acid (C18:1) can be hampered. The well-established rat insulin-producing RINm5F cell line is the prototype of such a dedifferentiated cell type. In this cell line, the physiological sFFA palmitic acid (16:0) shows a significant toxicity. Surprisingly, the non-metabolizable analog palmitic acid methyl ester, the monounsaturated FFAs palmitoleic acid (16:1) and oleic acid lack toxicity, and the latter two FFAs can even protect these cells against toxicity induced by palmitic acid.[31,56–59] In this context, it is noteworthy that the free unbound concentration of oleic acid was shown to be around twice as high as that of palmitic acid.[36] The reason for the lack of toxicity is a defect in the enzymatic degradation of the uFFAs. The protection against palmitic acid toxicity by oleic acid is potentially a blockade of palmitic acid degradation by oleic acid in the β-oxidation, since the amount of H_2O_2 is significantly decreased upon exposure to a combination of both FFAs.[31] In analogy, the same phenomenon has also been reported for dedifferentiated human insulin-producing cell lines (e.g., 1.1B4).[60] Therefore, poorly differentiated cell lines are not suited for structure–activity analyses. In contrast, cells of the human EndoC-βH1 beta-cell line are very suited for analyses of the mechanisms underlying toxicity of both sFFAs and uFFAs,[14] and they reveal effects similar to those obtained in isolated human islets.[13] It remains to be seen whether other new beta-cell lines of human origin or iPS-derived human beta cells will prove to be helpful tools for lipotoxicity studies in the future.



SCHEME 1 Peroxisomal β-oxidation versus mitochondrial β-oxidation (left) and saturated (left) versus unsaturated (right) free fatty acid (FFA) metabolism. Left section: FFAs are converted to acyl-CoA-FFAs by acyl-CoA synthetases (ACS). The FFAs activated in this way can be transported via ATP-binding cassette transporters (ABC) into the peroxisomal β-oxidation or imported into the mitochondrial β-oxidation via the carnitine transport system, namely carnitine palmitoyltransferase 1 (CPT1), carnitine acyl carnitine transferase (CACT), and carnitine palmitoyltransferase 2 (CPT2). In the first step of peroxisomal β-oxidation, the enzyme acyl-CoA oxidase (A-CoA-Ox) transfers electrons via FAD (flavin adenine dinucleotide) to molecular oxygen (O_2), producing hydrogen peroxide (H_2O_2), which is detoxified by catalase (CAT). In mitochondria, however, the electrons are transferred via acyl-CoA dehydrogenase (A-CoA-D) and FAD to the electron transport chain (ETC). Subsequently, the enoyl-CoA-FFA is chain-shortened by two carbon atoms in both organelles in a comparable manner with the enzymes enoyl-CoA hydratase (E-CoA-H), hydroxyacyl-CoA dehydrogenase (H-CoA-D), and ketoacyl-CoA thiolase (K-CoA-T). Right section: after several β-oxidation cycles of an unsaturated FFA with an even-numbered double bond in the molecule, a cis-4-acyl-CoA-FFA is formed, which is oxidized at position two and three by A-CoA-D. Subsequently, 2,4-dienoyl-CoA reductase (2,4 D-CoA-R) further converts it to a trans-3-acyl-CoA-FFA. At this metabolic step, both fatty acids with an odd-numbered double bond and the previously rearranged FFA with an even-numbered double bond are transformed by the Δ3,Δ2-trans-enoyl-CoA isomerase (Δ3,Δ2 tr-E-CoA-I). Finally, the FFAs obtained are metabolized in the same way as the saturated FFAs after the first step of oxidation (see left section).

10 | GLUCOLIPOTOXICITY IN HUMAN BETA CELLS

The term "glucolipotoxicity"[61] has been coined to describe the combined toxicity of glucose and FFAs. Since high glucose concentrations alone do not cause significant toxicity to human EndoC-βH1 beta cells,[22] glucose acts only as a potentiator of FFA toxicity.[22] It is well known that long-chain FFAs (C16-C18) increase caspase-3 activity, sFFAs more so than uFFAs (Figure 1A).[14] Interestingly, a potentiation of toxicity by high glucose concentrations (25 mmol/L) has been observed only in the case of long-chain and very long-chain sFFAs but not of uFFAs (C18-C20) (Figure 1C,D).[22,62] This goes along with an increase of ER stress markers (CHOP and XBP1s), starting with stearate (C18:0) and strongly pronounced in the case of the very long-chain sFFAs (C19-C20).[22] It is at variance from the situation with uFFAs, in which such an increase of ER stress markers was not detectable.[22] Despite the high beta-cell toxicity of very long-chain uFFAs, no significant ER stress has been observed in contrast to the sFFAs,[14] demonstrating that ER stress is not a general mechanism of lipotoxicity. Consequently, it seems that long-chain and very long-chain sFFAs in contrast to uFFAs put an increased stress upon the insulin folding apparatus in the ER[63] in the presence of high glucose concentrations.

Thus, in the case of very long-chain sFFAs (C19-C20), the glucose molecule as such potentiates the toxicity, but in the case of physiological long-chain sFFAs (C16-C18) glucose potentiates the toxicity only indirectly over a prolonged time span after initial conversion into palmitic acid (C16:0) through lipogenesis in the adipose tissue.

It can therefore be concluded that the constellation of long-chain sFFAs (i.e., hyperlipidemia) in the presence of high glucose concentrations (i.e., hyperglycemia), a situation of severe metabolic stress,[6] which accompanies insulinopenia and is therefore prevalent in advanced stages of T2DM, contributes significantly to beta-cell stress. It is causative for the deterioration of beta-cell function underlying the glucose intolerance in the T2DM scenario.[64,65] Thus, glucose can potentiate lipotoxicity under defined experimental circumstances,[22] but at variance from other views,[66] the glucose molecule itself is not the main driver of human beta-cell dysfunction and death under glucolipotoxic conditions. Obesity causing insulin resistance, which precedes the development of glucose intolerance and ultimately often results in T2DM manifestation, can provide the pathophysiological basis for lipid-mediated pancreatic beta-cell toxicity. When glucose is absorbed in the intestine after periods of starvation in quantities greater than those required to fill depleted glycogen stores in the liver, the surplus glucose is used for de novo lipogenesis in liver and palmitic acid is then directed into the adipose tissue where it is stored as triglyceride.[67] Since triglycerides generated from this principal hexose cannot be reconverted to glucose, they are transferred into the circulation as FFAs after release from triglycerides via lipoprotein lipase, first and foremost as palmitic acid in insulinopenia such as it exists during prolonged starvation and in the diabetic metabolic state. Thus, glucose acts in a beta-cell toxic manner ultimately as a FFA released from accumulated lipid stores in liver and adipose tissue in the same way as FFAs do after postprandial uptake in the intestine.

11 | THE ROLE OF LIPID DROPLET FORMATION IN LIPOTOXICITY IN HUMAN BETA CELLS

Lipid droplet deposition in the form of triglycerides under lipotoxic conditions is a well-known phenomenon in many mammalian cell types (i.e., liver and heart).[68–71] This comprises also pancreatic beta cells.[31,72–75]

However, available information on the role of lipid droplet formation in lipotoxicity is inconsistent. There is not only a clear difference in the sensitivity profile with respect to lipotoxicity among humans and rodents, but the ability to form lipid droplets in dependence on the FFA structure also differs. While, in rat beta cells, lipid droplets are formed upon exposure to long-chain and very long-chain uFFAs, sFFAs do not form lipid droplets.[75,76] In contrast, in human EndoC-βH1 beta cells both long-chain uFFAs and to a lesser extent long-chain sFFAs (C16-C19) form lipid droplets.[14] Surprisingly, however, the highly toxic very long-chain sFFAs (C20:0 and C22:0) do not form lipid droplets in human EndoC-βH1 beta cells.[14] Thus, it is evident that the profile of lipid droplet formation does not correlate very well with the lipotoxicity profile of the human EndoC-βH1 beta cells.

Interestingly, this difference between human and rat insulin-producing cells seems to be related to the presence of the enzyme stearoyl-CoA desaturase 1 (SCD1), which is only weakly expressed in murine beta cells but very pronounced in human beta cells.[22] Upon inhibition of SCD1, the human EndoC-βH1 beta-cell line exhibits increased lipotoxicity, while the ability to form lipid droplets through these sFFAs (C16:0-C19:0) is abolished resulting in a shift to a murine lipotoxicity profile.[21,22] Conversely, RINm5F rat beta cells develop reduced sensitivity and increased lipid droplet formation capacity after palmitic acid incubation following overexpression of human SCD1. Stearoyl-CoA desaturase 5 (SCD5), the second isoform of the enzyme in human beta cells, plays a minor role in FFA-mediated beta-cell toxicity and is most pronounced in the case of stearic acid (C18:0).[22]

Based on these findings, one might conclude that lipid droplets may have a protective function. However, when looking upon the structure–activity relationships, this conclusion needs to be revised. Arachidic acid (C20:0) and behenic acid (C22:0) are extremely toxic FFAs, which do not form lipid droplets, whereas the slightly weaker, but nevertheless also toxic nonadecanoic acid (C19:0), nonadecenoic acid (C19:1), gondoic acid (C20:1), and erucic acid (C22:1) lead to increased lipid droplet formation.[14] The same conclusion can be drawn with respect to the situation in combinations of FFAs. Combinations without beta-cell toxicity due to a high content of short- and medium-chain FFAs cause lipid droplet formation of an extent comparable to that of FFA combinations composed dominantly of beta-cell toxic long-chain FFAs.[14]

This underlines how important it is to analyze the mechanisms of toxic action in human beta cells using a wide range of FFAs as well as of FFA combinations rather than only of palmitic acid (C16:0) and oleic acid (C18:1) when considering the role of lipid droplet formation in lipotoxicity. On the other hand, it is difficult to envisage a clear advantage of lipid droplet deposition in a cell type such as the pancreatic beta cell, which is there for insulin storage and not for lipid storage.

12 | FUTURE THERAPEUTIC PERSPECTIVES

Weight reduction in persons with obesity can be achieved through restriction of calorie intake[77] and increased physical activity,[78] but sustained success is accomplished only in a minority of people in societies with a westernized life style. Prevention of overweight would be even better but realistically this is not achievable in such societies.

For treatment of gross overweight, bariatric surgery is meanwhile an established therapeutic option.[79,80] But this leaves the great majority of people with overweight and obesity unaided, although recent studies support the contention that weight loss clearly lowers the diabetes risk in people who are obese.[81]

For people being overweight with insulin resistance and hyperinsulinemia, therapeutic agents with a strong weight-reducing capacity will be required, but these agents should not significantly stimulate insulin secretion. Different is the situation in patients being obese with hypoinsulinemia due to a coexisting T2DM. These affected patients require a therapy with a compound such as semaglutide or

dulaglutide,[82] which in addition to their capacity to reduce body weight also increase insulin production and secretion through a trophic effect on the beta cells. Furthermore, desirable side effects such as cardioprotection are pleasant features of these compounds.[83] It will be interesting to see whether the pharmaceutical industry will be able to develop further drugs that can meet the therapeutic needs for the patients.[84] Individualized treatment of patients suffering from obesity requires agents or combinations of compounds with different therapeutic profiles for treatment of patients with or without T2DM. Importantly, these compounds should ideally be free of serious undesirable side effects such as gastrointestinal discomfort, which are often registered with certain forms of current treatment.[85]

13 | CONCLUSIONS

1. The analysis of structure–activity relationships is a potent tool for mechanistic studies. In the case of the toxicity of FFAs, this tool has proven to be of crucial importance for the elucidation of mechanisms underlying the toxicity to beta cells. Beyond the two principle physiological FFAs palmitic acid and oleic acid, dozens of structurally related FFAs are available for experimental studies. This is a huge advantage, since studies with palmitic acid and oleic acid alone have not provided conclusive insights into the mechanisms underlying FFA toxicity to beta cells.[9–11]

Very long-chain FFAs[46] and also *trans*-configured FFAs in excessive amounts[1] have been successfully banned over the last decades from the daily diet due to their inacceptable toxicity, and there is also a certain trend visible of opting for edible vegetable oils and fats with a greater content of medium- and short-chain FFAs, since they are not significantly toxic.[45] Monounsaturated FFAs in edible vegetable oils with a lower beta-cell toxicity are presently favorites of the consumer even though they are not completely free of any beta-cell toxic potential.[45]

2. It is the extraordinarily low antioxidative defense status due to the lack of catalase expression in the peroxisomes[4–7] that is responsible for the inability of the beta cell to cope with a toxic load of H_2O_2 generated in the peroxisomal β-oxidation.[14,20,30,31] Increased reactive oxygen species generation is thus a phenomenon constantly accompanying beta-cell lipotoxicity. This makes the vulnerable beta cell[7,8] so exceptionally susceptible to the toxic action of the FFAs.

Hence, any effort to minimize peroxisomal H_2O_2 generation and the resulting extreme toxicity of the subsequently generated hydroxyl radical in the pancreatic beta cell[6] is of crucial importance. This conclusion is not only supported by the proof of peroxisomal H_2O_2 generation in response to long-chain FFA exposure but also by the protection against palmitic acid toxicity provided by catalase expression in the peroxisomes of insulin-producing cells.[20,30]

In the long run raising the antioxidative defense, for example, through expression of an antioxidative enzyme such as catalase in the beta-cell peroxisomes and thereby improving the capacity for removal of toxic H_2O_2 produced in the beta cells without hampering the physiological insulin secretory capacity of the beta cell[86] may become an attractive new therapeutic option.

Progress in research thus allows the conclusion that lipotoxicity is a major cause for beta-cell dysfunction and death in the T2DM setting. The understanding of the pathomechanisms underlying pancreatic beta-cell toxicity will provide a good guide for the composition of a healthier diet with beta-cell protective potential.[45]

3. In an individual suffering from obesity, large triglyceride stores in its adipose tissue and in other organs are a result of excessive lipid and glucose consumption. The steadily increasing incidence of obesity in many countries, meanwhile also in children and young adults and often of excessive extent, can be compensated for many years in terms of maintenance of a beta-cell function sufficient to supply enough insulin to prevent mobilization of FFAs from lipid stores in the adipose tissue.

However, the insulin resistance typically associated with obesity and the emerging prediabetic phase as well as absolute insulin deficiency after manifestation of an open diabetic metabolic state result in a reduced efficiency of insulin in suppressing lipid storage in adipose tissue. This is the point in time, usually within the second half of the lifespan, when an increased FFA release from adipose tissue stores into the circulation emerges, which is capable of mediating lipotoxic effects in beta cells ultimately contributing to the steadily increasing worldwide incidence of T2DM.

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CONFLICT OF INTEREST STATEMENT

No conflict of interest statement.

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