Application for Support for the
Committee for Aid and Education in Neurochemistry (CAEN)

CATEGORY 1B: Research supplies for use in the applicant's home laboratory

Research Report Alberto Camacho Morales

Award started: March, 2013.

Award finished: July 2014.

The CAEN award support was highly beneficial to tackle two experiments in two research projects, one of them was accepted for publication in the Neurochemistry International Journal and the second one was accepted a month ago for publication to the Brain Research Journal.

Project 1)

Development of obesity requires a state of positive energy balance. Less evident is why the expansion of adipose tissue in obese individuals is strongly associated with insulin resistance and diabetes [12]. Two main hypotheses, not necessarily exclusive, have been formulated to explain this elusive link. One possibility is that changes in the repertoire of adipokines (e.g more leptin and resistin and less adiponectin) promotes insulin resistance [13]. The second hypothesis is that obesity leads to a failure of adipose tissue expandability and function that results in leakage of fatty acids from adipocytes and their ectopic accumulation in peripheral

metabolically relevant organs such as skeletal muscle, liver, beta cells or myocardium. The ensuing toxicity involves a process called lipotoxicity [14].

However, not only the amount but also the quality of the ectopically stored lipids is important, and that not all lipid species have the same toxic potential. Lipotoxic insult might affect intracelular organels and plasma membrane domains, such as lipid rafts. LR are small (10-200 nm), highly specialized, heterogeneous structures formed by sterol- and sphingolipid-enriched membranes containing saturated fatty acids. LRs are capable of compartmentalising proteins involved in specific signalling pathways [1]. Additionally, LR play an important role in trafficking and sorting of membrane proteins, as well as proteins involved in secretory and endocytic pathways [32, 33]. LR formation is promoted by saturated fatty acids. Incorporation of polyunsaturated fatty acids (PUFA) into the rafts may alter its size, composition and signalling function [1]. Another important consideration that lipid induced toxicity may be mediated through lipid induced protein modification. Specifically we refer to the nature of the lipid modification of proteins and their potential effects on protein targeting and functionality. Many studies indicate that acylation (mediated by saturated fatty acids) such as palmitoylation, myristoylation or formation of glycosylphosphatidylinositol (GPI)-anchors can target proteins to LR and promote specific signalling activation [34, 35]. Thus, the type of lipids might also be important in modulating raft functionality and might have important nutritional/therapeutic implications.

Altered integrity of LR in several organs has been suggested to contribute to the pathogenesis of diabetes. Supporting this possibility there is evidence that diets enriched in saturated fatty acids promote inflammation and insulin resistance [2-5]. Also cytokine induced insulin resistance (e.g. TNF-induced insulin resistance) in adipocytes results in accumulation of the GM3 ganglioside and decreased the number of insulin receptors within the caveolae microdomains, a characteristic structure of LR predominantly located in peripheral organs [43]. In addition, diet induced obesity disrupts the Cbl/CAP/TC10 raft insulin-signalling cascade in visceral adipose tissue [6].

In summary, LRs are important modulators of metabolic signals and that their integrity and functionality may be important for body energy homeostasis. We investigated whether obesity associated with high fat diet in mice or lipotoxic insult induced by palmitic acid stimulation to hypothalamic cell line results in alteration in protein recruitment in LRs microdomains and insulin resistance generation.

We found that genetic obesity increases recruitment of the IR negative regulator TANK-binding kinase 1 (TBK1) into LRs and PSD fraction. In vitro studies showed that incubation with saturated palmitic acid but not with unsaturated docosahexaenoic acid (DHA) or palmitoleic acid decreases association of IR and AKT and increases TBK1 recruitment into LRs and PSD domains, emulating what happens in the obese mice. TBK1 recruitment to insoluble domains correlates with decreases of IR tyrosine phosphorylation and ser473 AKT phosphorylation, markers of insulin resistance. These data support the hypothesis that

hyperlipidemia associated with genetic obesity alters targeting of TBK1 and insulin signaling proteins into insoluble LRs domains.

In particular, CAEN funding was helpfull to perfom LRs isolation from plasma membrane of in vivo and in vitro models.

Reference: Genetic obesity alters recruitment of TANK-binding kinase 1 and AKT into hypothalamic lipid rafts domains. Delint-Ramirez I, Maldonado Ruiz R, Torre-Villalvazo I, Fuentes-Mera L, Garza Ocañas L, Tovar A, Camacho A. Neurochem Int. 2015. 80:23-32.

Project 2)

The hypothalamus is an important target of lipids toxicity during obesity (Williams, 2012). High fat diet (HFD) intake in mice promotes lipotoxicity by activating ER stress and mitochondrial dysfunction resulting in insulin resistance and T2DM (Serra et al., 2013; Williams, 2012). As we pointed out ceramides, acylcarnitines and diacylglycerols are deleterious in compare to triglycerides in liver, adipose tissue, muscle and also brain (Camacho et al., 2012; Camacho et al., 2013; Medina-Gomez et al., 2007). In vitro evidence has shown that stimulation with the saturated lipid, palmitic acid, promotes mitochondrial deregulation, oxidative stress and JNK and NF-kB activation in muscle cells resulting in insulin resistance (Nie et al., 2014). Also, palmitic promotes ER stress activation in different cell types including neurons (Kwon et al., 2014; Nie et al., 2014; Yuzefovych et al., 2012).

These evidences suggest that ER and mitochondria are primary targets of lipids leading to insulin resistance states and the development of metabolic disorders including obesity and T2DM.

The ER and mitochondria are functionally and metabolically associated through a region known as mitochondria-associated membranes (MAMs) (Giorgi et al., 2015; van Vliet et al., 2014). Mitochondria and ER crosstalk into MAMs domains are maintained in part by mitofusin 2 (Mfn2) protein. Mfn2 is a membrane protein that regulates ER homeostasis coupling to mitochondrial activity coordinating body energy homeostasis (Giorgi et al., 2015). Disruption of MAMs integrity might result in ER stress activation and mitochondria dysfunction leading to metabolic failure (van Vliet et al., 2014). In fact, Mfn2 ablation in skeletal muscle or liver of mice modifies mitochondria morphology and function resulting in the generation of ROS and insulin resistance (Sebastian et al., 2012). Also, Mfn2 ablation in liver activates JNK and ER stress leading to insulin resistance (Sebastian et al., 2012). Of note, deletion of Mfn2 in anorectic POMC neurons of the arcuate nucleus promotes disruption of ER-mitochondria contacts, ER stress activation, leptin resistance and obesity (Schneeberger et al., 2013). Conversely, Agrp-specific Mfn2 knockout mice gained less weight when fed a high fat diet presumably due to decreased fat mass (Dietrich et al., 2013). These evidences suggest that Mfn2 seems to play a fundamental role in the ER-mitochondria crosstalk in metabolic relevant organs coordinating metabolic homeostasis by insulin or leptin sensitivity.

Here we determined whether saturated lipids coordinate hypothalamic Mfn2 expression, ER stress and its effect on insulin sensitivity using in vivo and in vitro models.

Our results found that lipotoxic stimulation induced by palmitic acid, but not the monounsaturated palmitoleic acid, decreases Mfn2 protein levels in hypothalamic mHypoA-CLU192 cells. Also, palmitic acid incubation activates ER stress response evidenced by increase in the protein levels of GRP78/BIP marker at later stage than Mfn2 downregulation. Additionally, we found that Mfn2 alterations induced by palmitic, but not palmitoleic, stimulation exacerbate insulin resistance in hypothalamic cells. Insulin resistance induced by palmitic acid is partially prevented by preincubation of the anti-inflammatory and the ER stress release reagents, sodium salicylate and 4 phenylbutirate, respectively. Finally, we demonstrated that lipotoxic insult induced by high fat feeding to mice alters Mfn2 proteins levels in arcuate nucleus of hypothalamus. Our data indicates that saturated lipids modulate Mfn2 expression in hypothalamus coordinating the ER stress response and the susceptibility to insulin resistance stage.

In particular, CAEN funding was helpfull to perfom the molecular characterization of ER stress activation and Mfn2 expression evidenced by western blot analysis.

Reference: Saturated lipids decrease mitofusin 2 leading to endoplasmic reticulum stress activation and insulin resistance in hypothalamic cells. Brenda Diaz, Lizeth Fuentes-Mera, Armando Tovar, Teresa Montiel, Lourdes Massieu, Herminia Guadalupe Martínez-Rodríguez and Alberto Camacho. Brain Reseacrh. 2015. 1627:80-9.

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Financial Status

Date	Amount	Consept	*Expenses*
			$ 4,998.47
	$ 23.44	Grant transfer	**$ 4,975.04**
03/07/13	$ 1,595.78	High fat and Chow diet. Cat. D12450B and D12492. Research Diets. New Jersey.	**$ 3,379.25**
17/07/13	$ 1,189.16	Regents	**$ 2,190.09**
30/07/13	$ 177.22	Regents	**$ 2,012.87**
08/20/2013	$ 1,748.61	Regents	**$ 264.27**
10/12/13	$ 146.82	Regents	**$ 117.44**
07/05/14	$ 110.36	Dr. Alberto Camacho Morales / Gastos varios	**$ 7.09**
	$ 7.09	Transfer fee	**$ 0.00**