PKC-β activation and pharmacologically induced weight gain during antipsychotic treatment

"...the inhibition of PKC-β could prevent or delay the development of obesity and obesity-related disorders introducing the hypothesis that the inhibition of PKC-β could be useful in therapy with APDs..."

KEYWORDS: adipose derived stem cells ■ adipose tissue ■ atypical antipsychotics ■ metabolic syndrome ■ PKC-β

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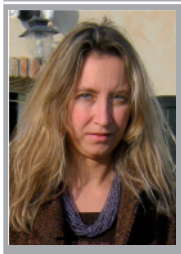

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Atypical antipsychotics (APDs) are now the most widely used drugs in the treatment of several mental disorders such as schizophrenia, bipolar disorder and other severe behavioral disturbances [1]. Thanks to great patient benefits such as the reduction of serious side effects, for example extrapyramidal symptoms, including tardive dyskinesia and heart arrhythmias [2], APDs are replacing the traditional antipsychotics, butyrophenones and phenothiazines.

In recent years, however, APDs have been found to exert a very strong impact on body metabolism, leading to the development of severe obesity, dyslipidemia and changes in insulin sensitivity, which are major risk factors associated with the development of cardiovascular complications [3].

The WHO considers this as a problem of global importance, not only because metabolic syndrome is a major cause of death in the general population, but also because it affects patients treated with APDs; up to 60% of deaths in this group are caused by this disease (with a mortality ratio of 1:57, compared with the general population) [4,5].

There is still uncertainty regarding the mechanism by which APDs can lead to severe weight gain and all of its effects, most notably the development of diabetes.

In this article, we present evidence highlighting the involvement of the β isoform of the protein kinase C family (PKC-β) in this unfortunate side effect of APDs related to weight gain [6].

First, we have to keep in mind that high glucose levels resultant of PKC-β activation, drive mesenchymal stem cells from both adipose tissue (adipose derived stem cells [ADSc]) and muscle (muscle derived stem cells [MDSCs]) towards an adipogenic potential in a reactive oxygen species (ROS)-dependent mechanism [7].

More importantly, in a recent study Pavan and colleagues investigated if PKC-β was involved in APD-related adipogenic events [8]. In their experimental design the researchers assumed that the increase in body fat could involve both the increasing lipid accumulation and the enhancement of preadipocyte differentiation. To this end, most APDs frequently used in the clinic such as aripiprazole, clozapine, quetiapine and risperidone were investigated in terms of their effects on proliferation and differentiation of ADSc and MDSCs.

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The results indicated that when MDSCs are exposed to external stimuli of an adipogenic lineage (such as the presence of a high glucose environment), the presence of APDs induces a remarkable increase in adipogenic transformation. Interestingly, APD treatment only affected transdifferentional events of ADSc and MDSCs on adult adipose cells. This APD-activated adipogenic differentiation program leading to the full conversion of muscle stem cells to adipocytes, as verified by molecular criteria, has never previously been described.

Regarding the signals driving this differentiation program, data suggests a crucial role for PKC-β, as revealed by the strong inhibitory effect of a specific PKC-β inhibitor (hispidine) and through its genetic downregulation by siRNA.

The direct involvement of PKC-β in adipocytes commitment was confirmed by a morphological evaluation in which a clear translocation of PKC-β from the cytosol to the plasma

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membrane (where it is then activated [9]) occurred only in the presence of APDs. This event was strongly related to the well known cellular response to high glucose which induces an increase in ROS production. In fact, ROS, through downstream effectors and in particular via PKC-β, leads to the neoformation of adipose cells. Thus, weight gain associated with APD use is owing not only to preadipocytes derived from adipose tissue, but also those derived from muscle tissue [8].

Interestingly, in a series of elegant works by Metha *et al.*, the involvement of PKC-β in adipose tissue homeostasis has been highlighted. In particular, Bansode and colleagues concluded that PKC-β deficiency increases fatty acid oxidation and reduces fat storage [10]. In their study the authors uncovered a role for protein PKC-β in triglyceride homeostasis by studying the consequences of a targeted disruption of this kinase. PKC-β mutant mice were considerably leaner and the size of white fat deposits was markedly decreased compared with wild-type littermates.

Moreover, triglyceride content in the liver and skeletal muscle of PKC-β null mice was significantly low. Interestingly, PKC-β null animals were hyperphagic and exhibited higher food intake and reduced feed efficiency versus wild-type mice [11].

To investigate the role of PKC-β signaling in metabolic adaptations to severe dietary stress, Metha's group studied the impact of a high fat diet (HFD) on PKC-β expression and the effect of PKC-β deficiency on profound weight gain [12]. Huang *et al.* reported that HFD selectively increased PKC-β expression in obesity-prone C57BL/6J mice, specifically in white adipose tissue (WAT) [12]; the expression levels were little or unchanged in the liver, muscle, kidney and heart. Basal PKC-β expression was also found to be elevated in WAT of obese mice (*ob*/*ob* mice). Remarkably, mice lacking PKC-β were resistant to HFD-induced obesity and demonstrated significantly reduced WAT and slightly higher core body temperatures. Unlike lean lipodystrophic mouse models, these mice did not have fatty livers nor did they exhibit insulin resistance. These observations suggest that PKC-β deficiency induced a unique metabolic state congruous with obesity resistance, thus raising the possibility that dysregulation of PKC-β expression could contribute to dietary fat-induced obesity and related disorders.

In conclusion, Huang's study has revealed a novel role for PKC-β in diet-induced obesity. It thus appears reasonable to propose that PKC-β is required in a signaling pathway that facilitates energy storage in response to a HFD, in part by triggering biological responses designed to paradoxically increase the efficiency of energy storage [12]. This would represent an example of the 'thrifty gene' phenotype and represent an advantage during times of food shortage [13].

The protection from obesity involves elevated oxygen consumption/energy expenditure and increased fatty acid oxidation in adipose tissue with concurrent increased mitochondria genesis [14]. The involvement of PKC-β in regulating mitochondrial biogenesis is supported by a recent observation that linked PKC-β to mitochondrial survival pathways [15].

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Taken together, these data indicate that one of the consequences of PKC-β deficiency is to partition fat towards increased oxidation and that the elevation in fatty acid oxidation capacity is powerful enough to overcome increased food intake. It is not clear whether the increased fatty acid oxidation in adipose tissue is sufficient to significantly promote the redistribution of fat from the liver and muscle to the adipocytes, much as fat transplantation does in fat-deficient lipodystrophic mice [16]. It is also conceivable that central regulatory actions of PKC-β in the brain, especially in the hypothalamus, may impact on body energy expenditure and metabolic control. Various CNS-mediated gene targets result in a lean phenotype, often with an increased metabolic rate [17]. In any event, PKC-β-dependent increase in oxidative capacity may have important therapeutic implications for the treatment of obesity and obesity-related disorders. In support of this notion, PKC-β antagonists are currently undergoing clinical trials to reduce diabetes-linked complications [18,19].

However, in times of feast and sedentary lifestyles, such as today, PKC-β appears to provide a survival disadvantage, causing an epidemic of hypertrophic obesity. It is possible that the upregulation of PKC-β expression and desensitization of insulin signaling in accumulated fat may serve as one of the mechanisms for maintaining obesity. Another intriguing finding is that few insulin signaling mediators have been reported to be diet-regulated at the transcriptional level in adipose tissue [20]. With their study, Shimomura *et al.* provide the first evidence that an insulin signaling kinase, PKC-β, is physiologically regulated at the transcriptional level in adipose tissue

by dietary fat [20]. Their data provides support for the hypothesis that adipose PKC-β is controlled specifically by factors responding to the consumption of dietary fat and that the expression of PKC-β is linked to development of obesity.

In light of such important findings we can address an important aspect of cell metabolism related to the effect of antipsychotics demonstrating a potential therapeutically relevant improvement.

Indeed, the inhibition of PKC-β could prevent or delay the development of obesity and obesity-related disorders introducing the hypothesis that the inhibition of PKC-β could be useful in therapy with APDs and thus may pave the way for new biochemically designed therapeutic approaches to treat these disorders.

However, much work remains to be done. First of all, *in vivo* validation demonstrating that PKC-β null and wild-type mice treated with PKC-β inhibitors are protected from APD-induced weight gain and yet retain their ability to counteract anxiety (otherwise, what is the benefit of preventing weight gain if APDs are also inhibited from performing their main function?), as well as defining the detailed mechanism by which APDs are activating PKC-β is needed.

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Bibliography

1 Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Hsiao JK: Effectiveness of antipsychotic drugs with chronic schizophrenia. *N. Engl. J. Med.* 353, 1209–1223 (2005).

2 Lean ME, Pajonk FG: Patients on atypical antipsychotic drugs: another high-risk group for Type 2 diabetes. *Diabetes Care* 26, 1597–1605 (2003).

3 Gebhardt S, Haberhausen M, Heinzel-Gutenbrunner M *et al.*: Antipsychotic-induced body weight gain: predictors and a systematic categorization of the long-term weight course. *J. Psychiatr. Res.* 43, 620–626 (2009).

4 Osby U, Correia N, Brandt L *et al.*: Mortality and causes of death in schizophrenia in Stockholm county, Sweden. *Schizophr. Res.* 45, 21–28 (2000).

5 Kim SF, Huang AS, Snowman AM, Teuscher C, Snyder SH: Antipsychotic drug-induced weight gain mediated by histamine H1 receptor-linked activation of hypothalamic AMP-kinase. *Proc. Natl Acad. Sci. USA* 104, 3456–3459 (2007).

6 Giorgi C, Agnoletto C, Baldini C *et al.*: Redox control of protein kinase C: cell and disease specific aspects. *Antioxid. Redox Signal.* 7, 1051–1085 (2010).

7 Aguiari P, Leo S, Zavan B *et al.*: High glucose induces adipogenic differentiation of muscle-derived stem cells. *Proc. Natl Acad. Sci. USA* 105, 1226–1231 (2008).

8 Pavan C, Vindigni V, Michelotto L *et al.*: Weight gain related to treatment with atypical antipsychotics is due to activation of PKC-β. *Pharmacogenomics J.* 10, 408–417 (2009).

9 Weiden PJ, Mackell JA, McDonnell DD: Obesity as a risk factor for antipsychotic noncompliance. *Schizophr. Res.* 66, 51–57 (2004).

10 Bansode RR, Huang W, Roy SK, Mehta M, Mehta KD: Protein kinase C-β deficiency increases fatty acid oxidation and reduces fat storage. *J. Biol. Chem.* 283, 231–236 (2008).

11 Matsumoto M, Ogawa W, Akimoto K *et al.*: PKCλ in liver mediates insulin-induced SREBP-1c expression and determines both hepatic lipid content and overall insulin sensitivity. *J. Clin. Investig.* 112, 935–944 (2003).

12 Huang W, Bansode R, Mehta M, Mehta KD: Loss of protein kinase C-β function protects mice against diet-induced obesity and development of hepatic steatosis and insulin resistance. *Hepatology* 49, 1525–1536 (2009).

13 Neel JV: Diabetes mellitus: a 'thrifty' genotype rendered detrimental by 'progress'? *Bull. World Heath Organ.* 77, 694–703 (1999).

14 Oberkofler H, Klein K, Felder TK, Krempler F, Patsch W: Role of peroxisome proliferator-activated receptor-γ coactivator-1α in the transcriptional regulation of the human uncoupling protein 2 gene in INS-1E cells. *Endocrinology* 147, 966–976 (2006).

15 Pinton P, Rimessi A, Marchi S *et al.*: Protein kinase C β and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science* 315, 659–663 (2007).

16 Gavrilova O, Marcus-Samuels B, Graham D *et al.*: Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J. Clin. Investig.* 105, 271–278 (2000).

17 Reitman ML: Metabolic lessons from genetically lean mice. *Annu. Rev. Nutr.* 22, 459–482 (2002).

18 Nishizuka Y: The molecular heterogeneity of protein kinase C and its implications for cellular regulation. *Nature* 344, 661–665 (1998).

19 Blobe GC, Khan WA, Halpern AE, Obeid LM, Hannun YA: Selective regulation of expression of protein kinase C β isoenzymes occurs via alternative splicing. *J. Biol. Chem.* 268, 10627–10635 (1993).

20 Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL: Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and *ob/ob* mice. *Mol. Cell* 6, 77–86 (2000).