Distinct roles of specific fatty acids in cellular processes: implications for interpreting and reporting experiments

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Watt MJ, Hoy AJ, Muoio DM, Coleman RA. Distinct roles of specific fatty acids in cellular processes: implications for interpreting and reporting experiments. Am J Physiol Endocrinol Metab 302: E1–E3, 2012. First published November 15, 2011; doi:10.1152/ajpendo.00418.2011.— Plasma contains a variety of long-chain fatty acids (FAs), such that about 35% are saturated and 65% are unsaturated. There are countless examples that show how different FAs impart specific and unique effects, or even opposing actions, on cellular function. Despite these differing effects, palmitate (C16:0) is regularly used to represent “FAs” in cell based experiments. Although palmitate can be useful to induce and study stress effects in cultured cells, these effects in isolation are not physiologically relevant to dietary manipulations, obesity, or the consequences of physiological concentrations of FAs. Hence, authors should avoid conclusions that generalize about “FAs” or “saturated FAs” or “high-fat diet” effects if only a single FA was used in the reported experiments.

FATTY ACIDS (FAs) derived from the lipolysis of adipose tissue triacylglycerol are released into the circulation, where they can be taken up by cells to be used as an energy substrate or to form other lipids that are essential for survival. However, there is now a compelling body of evidence that chronic oversupply of FAs to nonadipocytes can result in cellular dysfunction and even apoptotic cell death. Right? Well, almost. Although on face value this statement appears to be accurate, as a generalization, the statement overlooks and even misrepresents the distinct roles of specific FAs in cellular processes and physiological functions.

Plasma contains a variety of long-chain FAs, such that about 35% are saturated and 65% are unsaturated. There are countless examples that show how different FAs impart specific and unique effects, or even opposing actions, on cellular function. Despite these differing effects, palmitate (C16:0) is regularly used to represent “FAs” in cell based experiments. This approach is problematic because palmitate often induces cyto-toxic responses that are at variance with most other FAs. In fact, it is observed in many cases that the addition of an equal concentration of oleate prevents the adverse effects of palmitate. Thus, it is incorrect to incubate cells with palmitate in the absence of other unsaturated FAs and infer that the outcome represents a physiological effect of “FAs” or of “saturated FAs.” In skeletal muscle, for example, palmitate induces diacylglycerol and ceramide accumulation (1, 2), stress kinase activation (13, 18), endoplasmic reticulum stress (9), proinflammatory signaling and cytokine production (4, 5, 16), mitochondrial reactive species production (18), and apoptosis (15) (Fig. 1). By contrast, oleate has little or no effect on these processes and even prevents the stress or the toxic effects of palmitate when they are coincubated (2, 7, 15). Similar reports exist for almost all cell types. For example, long-chain saturated FAs (C14:0–C18:0) activate Toll-like receptor 4 signaling in macrophages, but preincubation with polyunsaturated FAs attenuates this response (12). Similarly, palmitate activates the NLRP3 inflammasome in macrophages, whereas oleate has no effect (17). Differences between species are also observed when radiolabeled FAs are used to measure rates of FA oxidation and storage. The choice of a representative FA influences the interpretation of such experiments (3, 11), as does the addition to the incubating medium of l-carnitine, which permits normal rates of mitochondrial uptake and oxidation of long-chain FA (data not shown). However, it is appropriate to use a single FA, including palmitate, to examine relative amounts of uptake, oxidation, and/or incorporation in cells treated acutely under specific conditions.

Although palmitate can be useful to induce and study stress effects in cultured cells (6), these effects in isolation are not physiologically relevant to dietary manipulations, obesity, or the consequences of physiological concentrations of FAs. To avoid mistaken interpretations, we recommend that FAs should be used at physiological concentrations (e.g., 50–750 μM), that FAs should be complexed to albumin at an appropriate molar ratio (0.5:3 fatty acid/albumin), and that the interpretation of an experiment should be limited to the experimental condition employed. Investigators should not infer that the effect of a single FA tells one something about “FA effects” in general, particularly if coinucubation with an equal amount of oleate rescues normal cellular function. Where possible, investigators should endeavor to replicate key experiments with a mixture of saturated and unsaturated FAs delivered in a molar ratio and in concentrations that represent those found in blood (e.g., 1:1 or 1:2 palmitate/oleate; 1:2:1 palmitate/oleate/linoleate) (8). Such studies should be construed as crucial controls that provide a minimum standard to judge the general role of “FAs” in a physiological context (Table 1). Although this stance might be considered hypercritical or bordering on just plain finicky, the devil is often in the details; accurate reporting and careful, inclusive experiments can change the interpretation of a data set and, ultimately, the conclusions relating to biological function.

These interpretations apply in vivo. To take one example, Obici et al. (8) and Ross et al. (10) conducted a seminal series...
of studies to examine the effects of central FA administration on hepatic glucose metabolism and feeding. In the first study (8), the authors showed that infusing oleate \((C18:1)\), but not octanoate \((C8:0)\), into the third cerebral ventricle enhanced insulin action, inhibited hepatic glucose production and decreased food intake. Those authors accurately titled the study “Central Administration of Oleic Acid Inhibits Glucose Production and Food Intake”; however, they inaccurately concluded that “This is the first demonstration that fatty acids can signal nutrient availability to the CNS.” Later studies published in *AJP-Endocrinology and Metabolism* (10) confirmed that hepatic insulin action is enhanced by central oleate administration but also showed that palmitate replicated these effects, albeit with considerably less potency than oleate, and that linoleate \((18:2\alpha\omega6)\) had no effect. In this case, the title “Differential effects of hypothalamic long-chain fatty acid infusions on suppression of hepatic glucose production” describes the effects more precisely than does the original publication.

Thus, to ensure accurate reporting and interpretation, we recommend that authors identify the specific FA used in the study if a single FA is used, that they provide the FA and albumin concentrations in the methods sections of their articles, that they use a mixture of saturated and unsaturated FAs where possible, and, importantly, that they avoid conclusions that generalize about “FAs” or “saturated FA’s” or “high-fat diet” effects if only a single FA was used in the reported experiments.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

A.J.H. prepared the figure; M.J.W. drafted the manuscript; M.J.W., A.J.H., D.M.M., and R.A.C. edited and revised the manuscript; M.J.W., A.J.H., D.M.M., and R.A.C. approved the final version of the manuscript.

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