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### **Pleiotropic mechanisms of action of perhexiline in heart failure**

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5 **Pleiotropic mechanisms of action of perhexiline in heart failure**

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**Abstract**

*Introduction:* The re-purposing of the anti-anginal drug perhexiline (PHX) has resulted in symptomatic improvements in heart failure (HF) patients. The inhibition of carnitine palmitoyltransferase-1 (CPT-1) has been proposed as the primary mechanism underlying the therapeutic benefit of PHX. This hypothesis is contentious.

*Areas covered:* We reviewed the primary literature and patent landscape of PHX from its initial development in the 1960s through to its emergence as a drug beneficial for HF. We focused on its physico-chemistry, molecular targets, tissue accumulation and clinical dosing.

*Expert opinion:* Dogma that the beneficial effects of PHX are due primarily to potent myocardial CPT-1 inhibition is not supported by the literature and all available evidence point to it being extremely unlikely that the *major* effects of PHX occur via this mechanism. *In vivo* PHX is much more likely to be an inhibitor of surface membrane ion channels and also to have effects on other components of cellular metabolism and reactive oxygen species (ROS) generation across the cardiovascular system. However, the possibility that *minor* effects of PHX on CPT-1 underpin disproportionately large effects on myocardial function cannot be entirely excluded, especially given the massive accumulation of the drug in heart tissue.

**Keywords:** perhexiline, carnitine palmitoyltransferase, heart failure, metabolism, modulation, therapy

**Article highlights**

- In contrast to recent dogma, we propose that it is unlikely that the therapeutic benefit of perhexiline in patients with HF is due, in large part, to the inhibition of CPT-1.
- All available data suggests that PHX is a drug of low potency, low specificity and low selectivity that interacts with multiple surface membrane ion channels and other intracellular components that impinge on cellular metabolism and the generation of reactive oxygen species (ROS).
- The possibility that *minor* effects of PHX on CPT-1 underpin disproportionately large effects on myocardial function cannot be excluded, especially given the massive accumulation of PHX in myocardial tissue.
- A scheme is described that considers the pleiotropic effects of PHX via the inhibition of surface membrane ion channels and its accumulation in mitochondrial membranes.
- The combined action of PHX on multiple targets throughout the cardiovascular system probably underpins the symptomatic improvements in HF patients.

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3 **(1) Introduction- new therapies for heart failure (HF).**  
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5 As defined by Yancy and colleagues “HF is a complex clinical syndrome that results from  
6 any structural or functional impairment of ventricular filling or ejection of blood”.<sup>1</sup> It is a global  
7 epidemic, affecting 10% of individuals over the age of 65 years and its clinical progression  
8 results in mortality of 50% within 4 years of diagnosis.<sup>1</sup> Contemporary approaches, which  
9 aim to correct left ventricular ejection fraction (LVEF) and halt or normalize the progression  
10 of myocardial dysfunction are sub-optimal and involve the modulation of neurohumoral  
11 targets including  $\beta$ -adrenergic ( $\beta$ -AR) blockers, suppression of the renin-angiotensin-  
12 aldosterone (RAA) system (angiotensin II receptor (AT1R), angiotensin-converting enzyme  
13 (ACE), aldosterone antagonism), vasodilators and diuretics. Whilst these conventional  
14 therapies primarily modulate upstream regulators of the circulatory system, there are newer  
15 strategies that aim to target more ‘myocardially-focused’ elements including the drivers of  
16 progressive contractile and arrhythmogenic events that occur *within* cardiac cells<sup>2-5</sup> and even  
17 approaches that replace or repair damaged myocardium.<sup>6</sup>  
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31 We have previously described normal myocardial cell signaling in terms of the  
32 synchronization of coupled systems.<sup>7-9</sup> For example, there is functional integration between  
33 cellular ion fluxes and metabolism in which the dynamic cycling of intracellular  $\text{Ca}^{2+}$  is  
34 critically modulated by ATP-dependent processes, and many events in ATP synthesis are  
35  $\text{Ca}^{2+}$ -dependent. Consequently, the functional deterioration of the myocardium in long-term  
36 cardiac diseases such as HF is associated with the progressive desynchronization within  
37 and between numerous linked processes. Using the example above, disease-linked  
38 derangement in  $\text{Ca}^{2+}$  signaling, which is considered a major feature of HF pathogenesis,  
39 impacts on ATP bioavailability and vice versa.<sup>7, 8</sup>  
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50 If we consider then that approaches that aim to correct HF-linked  $\text{Ca}^{2+}$  signaling  
51 dysfunction<sup>7, 8, 10-14</sup> may positively influence other ‘linked’ systems such as metabolism, there  
52 is substantial merit in developing new therapies that alter cellular metabolism with a view to  
53 rescuing cellular  $\text{Ca}^{2+}$  handling. An emergent approach to normalizing cardiac performance  
54 in HF therefore is the direct modulation of the myocardial metabolic state and drugs that  
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3 promote a switch in substrate utilization from fatty acid oxidation (FAO) to glycolysis (so-  
4 called 'metabolic modulators') are keenly sought.<sup>15-18</sup>

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7 Important problems in developing new therapeutic approaches though include the  
8 limitations of rational drug design and sometimes prohibitory regulatory and financial barriers  
9 which have led to creative solutions for finding new niches for existing drugs in the clinical  
10 landscape.<sup>4, 19-23</sup> 'Repositioning', 'repurposing' and 'reappropriating' have become buzzwords  
11 in the contemporary drug development framework.

12  
13 In this article, we consider some of these issues relating to perhexiline (PHX; 2-(2,2-  
14 dicyclohexylethyl)piperidine), a drug reappropriated from the treatment of angina pectoris  
15 (AP) and reported to be a clinically useful drug in the management of HF. The history of  
16 PHX, from its initial development as an L-type Ca<sup>2+</sup> channel blocker<sup>24-29</sup>, its efficacy in AP<sup>29-</sup>  
17 <sup>35</sup>, its contraindications, withdrawals and now its emergence in treating HF has been  
18 comprehensively reviewed previously.<sup>15, 18, 36</sup> However, its mechanism of action remains  
19 contentious and here we consider, with specific reference to the patent landscape, how the  
20 apparent disconnect between the measureable clinical benefits of PHX in HF and the  
21 molecular underpinnings of its actions can be reconciled.

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38 **(2) Promoting the shift from FAO to glycolysis in HF – the rationale for carnitine**  
39 **palmitoyl transferase (CPT) inhibition.**

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41 The hallmark metabolic dysfunction in HF is the intrinsic shift from FAO (aerobic) to glucose  
42 (anaerobic) metabolism.<sup>37-42</sup> This transition is likely the consequence of a lack of adequate  
43 cardiac tissue oxygenation in the diseased state- although this is questionable in non-  
44 ischaemic HF (DCM)<sup>43</sup>- but it may, to some extent, represent an adaptatory mechanism to  
45 preserve myocardial function. Although glucose metabolism yields much less ATP than FAO  
46 (a theoretical yield of 38 moles ATP for the complete oxidation of glucose versus 129 moles  
47 ATP per mole of palmitate)<sup>44</sup>, it is a more oxygen efficient mode of energy production  
48 yielding 3.17 moles ATP/moles atomic oxygen versus 2.8 moles of ATP/moles atomic  
49 oxygen for FAO of palmitate.<sup>44</sup> Approaches that aim to promote this 'oxygen sparing' effect  
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3 would also be coupled to the reduced flux of intermediates through the tricarboxylic acid  
4 (TCA or Krebs cycle) which would decrease the generation of reactive oxygen species  
5 (ROS) and minimize lactate production thereby preventing acidosis (Figure 1). Potentially,  
6  
7 the combined effects of reduced ROS and normalized pH may contribute more to the  
8  
9 beneficial effect of 'metabolic modulation' than considerations regarding the lower net yield  
10  
11 of ATP. Since metabolism is enmeshed with numerous other cellular process (e.g.  
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13 mTORC1/AMPK-mediated nutrient sensing modules involved in cell growth and  
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15 autophagy)<sup>45-48</sup>, there are other considerations for 'metabolic modulation' beyond ATP  
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17 synthesis and consumption and energy substrate utilization.  
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21 Singh and colleagues have recently reviewed candidates for therapeutic metabolic  
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23 modulation including the TCA cycle, pyruvate dehydrogenase (PDH), malonyl CoA-  
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25 decarboxylase (MCD), fatty acid oxidation (FAO), insulin sensitivity and carnitine  
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27 palmitoyltransferase (CPT-1).<sup>18</sup> Of these, it is CPT-1, an outer-mitochondrial membrane-  
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29 resident enzyme, that has received the most attention. There are three isoforms of CPT-1  
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31 (1A, 1B and 1C); CPT1A and B share 63% sequence homology and are abundantly  
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33 expressed in a wide variety of tissues, whereas CPT-1C is restricted to the brain.<sup>49</sup> CPT-1B  
34  
35 is the predominant isoform in the heart and exhibits a lower affinity for its substrate carnitine  
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37 than CPT-1A (approximately 15-fold) but is more sensitive to inhibition by the endogenous  
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39 inhibitor malonyl co-A inhibition than CPT1A.<sup>49</sup> CPT-1 catalyzes the formation of acyl  
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41 carnitine, via the transfer of the acyl group of a long-chain fatty acyl-CoA from coenzyme A  
42  
43 to carnitine<sup>50</sup>. Acyl carnitine subsequently transfers from the cytosol into the mitochondrial  
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45 inter-membrane space to undergo CPT-2-catalyzed conversion back to acyl-CoA which  
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47 undergoes catabolic beta-oxidation in the mitochondrial matrix to yield acetyl-CoA which  
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49 then enters the TCA cycle (Figure 1). In principle therefore, CPT-1 inhibition, which *in situ*  
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51 mediated by of malonyl Co-A levels (Figure 1), reduces mitochondrial import and oxidation  
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53 of fatty acids, promotes glycolysis via reduced acetyl-CoA production and decreases ROS  
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55 and lactate accumulation (Figure 1) albeit at the expense of ATP production.  
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3 One approach to therapeutically target CPT-1 activity is the development of  
4 heterocyclic and piperidine compounds that inhibit malonyl-CoA decarboxylase (MCD) which  
5 leads to an accumulation of malonyl-CoA<sup>51, 52</sup>, the endogenous inhibitor of CPT-1 *in vivo*  
6 (Figure 1).<sup>53</sup> We return to the subject of malonyl-CoA inhibition of CPT-1, and the  
7 implications for adjunctive PHX-mediated inhibition, in section 4.  
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13 However, in the context of treating chronic myocardial dysfunction, it is the direct  
14 pharmacologic inhibition of CPT-1 that has captured the imagination. In a compelling series  
15 of clinical studies, PHX (hailed as the 'forerunner of metabolic agents'<sup>16</sup>) has been shown to  
16 produce beneficial effects in patients with refractory HF symptoms despite otherwise optimal  
17 medical therapy<sup>54</sup>, symptomatic hypertrophic cardiomyopathy (HCM)<sup>55</sup>, and non-ischaemic  
18 dilated cardiomyopathy (DCM).<sup>56</sup> Such symptomatic improvements include reduced New  
19 York Heart Association (NYHA) classification of disease severity<sup>54, 55</sup>, increased peak  
20 oxygen uptake (VO<sub>2</sub> max)<sup>54, 55</sup>, improvements in quality-of-life scores<sup>54, 56, 57</sup> and six-minute  
21 walk tests<sup>57</sup>. Whether these functional benefits are dependent on improved myocardial  
22 function (e.g. left ventricular ejection fraction; see<sup>54</sup> vs. <sup>56</sup>), or interestingly skeletal muscle  
23 function<sup>54</sup>, has yet to be fully established. Moreover, although these investigations evidence  
24 beneficial changes in the phosphocreatine/ATP ratio which suggest generalized  
25 improvements in cardiac energetics, in their study of DCM patients Beadle *et al.* failed to find  
26 evidence of altered cardiac substrate utilization<sup>56</sup>. This finding is at odds with the principal  
27 dogma of earlier studies on PHX that its main effect is to promote the shift from FAO to  
28 glycolysis.<sup>25, 29, 58, 59</sup> It is necessary therefore to harmonize the therapeutic benefit of PHX  
29 with its mechanism of action and in sections 3 to 6 we address this issue by reference to the  
30 patent literature and the physico-chemical determinants of CPT-1 inhibition.  
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### 52 **(3) PHX, CPT-1 and the patent landscape.**

53 Notwithstanding some of the disparate effects of PHX in patients with different forms of  
54 chronic heart disease<sup>54-56</sup>, an important question is to what extent does CPT-1 inhibition  
55 contribute to the clinical improvement measured in these patients. The first patent relating to  
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3 PHX and CPT-1 was filed by Horowitz and Kennedy<sup>60</sup> and sought to protect the use of a  
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5 screening assay for discriminating compounds on the basis of CPT-1 inhibition. Such  
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7 compounds were proposed to be useful in treating 'ischaemic conditions'.

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9 On the basis of demonstrating improved clinical endpoints in PHX-treated patients,  
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11 Frenneaux and colleagues have filed a suite of patents pertaining to its efficacy in ischaemic  
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13 and non-ischaemic HF<sup>61</sup>, HF with preserved ejection fraction (HFpEF)<sup>62</sup> and hypertrophic  
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15 cardiomyopathy (HCM).<sup>63</sup> These patents all include the phrase "Perhexiline (2-(2,2-  
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17 dicyclohexylethyl)piperidine) is a known anti-anginal agent that operates principally by virtue  
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19 of its ability to shift metabolism in the heart from free fatty acid metabolism to glucose, which  
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21 is more energy efficient", but it is striking that none of these patents claim that the clinical  
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23 benefit of PHX is because of CPT-1 inhibition.

24  
25 However, the CPT-1-centricity of the proposed mechanism of PHX action persists  
26  
27 and a recent patent filing from the University of Aberdeen seeks to protect intellectual  
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29 property (IP) around a fluorinated PHX-derivative useful for treating a very broad range of  
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31 "disorders that are ameliorated by the inhibition of carnitine palmitoyltransferase.....".<sup>64</sup>

32  
33 With regard to the progression of HF being a consequence of the deterioration of  
34  
35 linked systems (see section 1), it is interesting to note that the patent on PHX effect in  
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37 ischaemic and non-ischaemic HF<sup>61</sup> states that "metabolic manipulation with PHX is effective  
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39 in modifying not an inciting influence, but rather the common programme of the chronic heart  
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41 failure". It is plausible therefore that the metabolic dysfunction in HF is secondary to the  
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43 abnormality in some other linked system and that PHX may be useful in modifying the  
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45 chronic metabolic remodelling associated with HF progression and not in preventing disease  
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47 onset.

#### 51 52 ***(4) Is PHX a physiologically relevant CPT-1 inhibitor?***

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54 The symptomatic improvement in HF following PHX administration has been linked to a  
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56 broad range of phenomenological descriptions including cGMP-dependent potentiation of  
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58 platelet sensitivity to NO<sup>32, 35, 65</sup>, vasodilatory effects<sup>66, 67</sup>, positive inotropic actions via  
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3 troponin-C<sup>68</sup>, circulating levels of ROS<sup>69</sup> and insulin sensitization.<sup>70</sup> However, assigning the  
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5 molecular target(s) of PHX that underlies these phenomena has proved more difficult.  
6

7 The patent filing of Horowitz and Kennedy<sup>60</sup>, together with the companion paper from  
8  
9 the same group<sup>71</sup>, used an *in vitro* (intact) mitochondrial assay to robustly make the case  
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11 that PHX (and also amiodarone) was a CPT-1 inhibitor. It was here that the concept that the  
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13 “major biochemical basis of the anti-ischaemic effect of PHX is inhibition of CPT-1” was  
14  
15 born. Measuring drug IC<sub>50</sub> values in this type of assay is prone to influence from several  
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17 variables, including the levels of the protein in the tissue, local concentration of drug and the  
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19 accessibility of its binding site (i.e. isolated mitochondrial preparations). To our knowledge,  
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21 the binding affinity of PHX for CPT-1 has not been determined. We acknowledge that it is  
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23 difficult to experimentally mimic *in vitro* the precise conditions that may be important  
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25 determinants of drug-target interaction *in vivo*. Although we consider investigations using  
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27 recombinant CPT isoforms expressed in methylotrophic *Pichia pastoris* yeast to constitute  
28  
29 the most direct measurements of PHX-CPT interaction<sup>72, 73</sup>, the likelihood of PHX binding  
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31 multiple targets (1) makes it impossible at present to assign the relative contributions of PHX  
32  
33 modulation of individual targets to the downstream effects. As has been noted previously,  
34  
35 these issues relating to ‘holistic’ assessments of drug action are presently beyond the  
36  
37 resolution of contemporary assays.<sup>22</sup>  
38

39 On the basis of Kennedy’s early work on CPT1 inhibition by PHX<sup>71</sup>, the review  
40  
41 literature on PHX perpetuates the idea that the primary mechanism of action the drug is  
42  
43 through CPT-1 inhibition (for example, “the identification of CPT-1 as perhexiline’s major  
44  
45 biochemical site of inhibition”<sup>15</sup>). Given the paucity of supporting data, the evidence base for  
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47 this assertion is not convincing (Table 1).  
48

49 PHX shares common properties with other Na<sup>+</sup>-, K<sup>+</sup>- and Ca<sup>2+</sup>- ion channel blockers,  
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51 many of which are recognized anti-arrhythmic drugs (Table 2), including its appreciable  
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53 hydrophobicity and an ionizable nitrogen which is positively charged at neutral pH (7.4). In  
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55 this regard, PHX is chemically different to etomoxir, which is an exemplar CPT inhibitor<sup>49</sup>  
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57 and in a quasi-exhaustive review of CPT inhibitor chemistry, PHX, oxfenicine (where the  
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3 active metabolite is 4-hydroxyphenylglyoxylate (HPG)) and amiodarone were clearly  
4 categorized as “miscellaneous compound[s] reported to be CPT inhibitors”.<sup>49</sup> Ceccarelli *et al.*  
5 concluded that in view of PHX’s extremely weak inhibition of CPT-1 it was “highly  
6 questionable that the effects are due to CPT1 inhibition”.<sup>49</sup> The chemical properties of PHX  
7 would also suggest relatively poor selectivity and specificity.<sup>74</sup> This is corroborated by the  
8 data in Table 1 which emphasizes the multiplicity of PHX targets and importantly, all  
9 available data point to PHX exerting its *least* potent effect on CPT-1 (Table 1). However, it is  
10 recognized that functional pleiotropy (i.e. drug promiscuity), which we have previously  
11 considered as ‘magic shotguns’ versus ‘magic bullets’<sup>75</sup>, can be beneficial.<sup>76</sup> This is  
12 discussed this further in section 7.

13  
14 In consideration of the other reported targets of PHX (Table 1), the relevance of  
15 competitive inhibition of CPT-1 by PHX *in situ* (characterized by an IC<sub>50</sub> of 77 µM determined  
16 in isolated mitochondria membrane preparations<sup>71</sup>) may be questioned. Moreover, the  
17 inhibition of CPT-1 by malonyl-CoA is 10-100 times more potent (IC<sub>50</sub> of 0.6 - 6.3 µM  
18 depending on the local concentration of palmitoyl-CoA (25 µM or 150 µM, respectively))<sup>77</sup>  
19 with a recent *ex vivo* study suggesting that malonyl-CoA produces a 33% inhibition of CPT-1  
20 activity in resting (non-stimulated) muscle.<sup>77</sup> However, it may be important *in vivo* that the  
21 actions of PHX on CPT-1 are mechanistically different to those of malonyl co-A and  
22 oxfenicine (IC<sub>50</sub> of CPT-1 inhibition = 11 µM<sup>78</sup>) and it has been suggested that PHX acts at a  
23 protected mitochondrial site not amenable to proteolysis i.e. in the plane of the membrane.<sup>15,</sup>  
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71, 79 It is also intriguing that the specific counterion used in the preparation of the clinical  
formulation of a drug may have a profound impact on its physico-chemical properties,  
including logP.<sup>80</sup> This is especially pertinent for maleate which has been shown to directly  
influence cellular metabolism via promoting the utilization of glucose and the accumulation of  
fatty acids.<sup>81, 82</sup> The contribution of the maleate ‘counterion’ to the effects attributable to PHX,  
especially in the context of altered metabolism, warrants further investigation.<sup>83</sup>

Recent proteomic and metabolic studies have corroborated a complex effect of PHX  
on metabolism.<sup>84, 85</sup> In a study of mice treated for 4 weeks at steady-state PHX plasma

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3 concentration, Yin and colleagues reported the activation of the pyruvate dehydrogenase  
4 complex (PDH) (Figure 1) and a “rebalancing of carbon and nucleotide phosphate fluxes,  
5 fuelled by increased lactate and amino acid uptake, to increase metabolic flexibility and  
6 maintain cardiac output”.<sup>84</sup> Furthermore, Ceccarelli determined a greater potency of PHX on  
7 ketone body (KB) generation and beta-oxidation (FAO) inhibition in rat and human  
8 hepatocytes ( $IC_{50} = 14.8$  and  $22.4 \mu M$ , respectively) than on CPT-1 inhibition ( $IC_{50} > 100$   
9  $\mu M$ ).<sup>49</sup> Together these data advance the idea that the therapeutic effects of PHX may be  
10 due, in some part to *bona fide* metabolic modulation, but the notion of PHX being a potent  
11 inhibitor of CPT-1 needs further evaluation.  
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21 To this end, the effects of PHX on reduced ROS may be more attributable to direct  
22 inhibition of NADPH oxidase (NOX2) rather than effects on cardiac energetics *per se*.<sup>86</sup> Also,  
23 Unger and colleagues reported that in non-ischaemic working rat hearts, “perhexiline  
24 increases myocardial efficiency by a mechanism(s) that is largely or entirely independent of  
25 its effects on CPT”.<sup>87</sup>  
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### 33 **(5) PHX metabolism, physico-chemistry and tissue accumulation**

34 A well-known consequence of longer term (unmonitored) PHX administration (typically > 3  
35 months)<sup>88</sup> is lipodosis-induced hepato- and neuro-toxicity, hypoglycaemia and weight loss.<sup>31,</sup>  
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34, 89-94 However, there is now a more complete understanding of the risk factors  
predisposing individuals to adverse effects, including patient-specific CYP2D6 status.<sup>95-98</sup>  
Oxidative metabolism of PHX produces mono- and di-hydroxylated metabolites. The  
predominant metabolite detected in plasma - *cis*-4 hydroxyperhexiline - is found at  
concentrations higher than PHX.<sup>99, 100</sup> Those individuals with CYP2D6 insufficiency (‘poor  
metabolizers’) have a reduced capacity to form this metabolite<sup>101</sup> and thus PHX is  
contraindicated in these individuals. This association between drug chirality and toxicity is  
also important<sup>99, 100, 102</sup> since there is reported stereoselectivity of (+) and (-) enantiomers to  
metabolism.<sup>95, 100, 101</sup> The asymmetry of the C2 of the piperidine ring means that PHX is  
administered as a racemic mixture of (+) and (-) enantiomers. The (+)-PHX enantiomer is

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3 cleared more slowly from the plasma<sup>103-105</sup> and is associated with a more pronounced toxicity  
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5 profile.<sup>104, 106</sup> Sallustio's recent patent on the use of (-)-PHX seeks to negate the issue of  
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7 toxicity and other problems possibly linked to the complex mixture of different stereoisomers  
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9 and metabolites of unknown efficacy arising from administration of the unresolved racemic  
10  
11 mixture.<sup>106, 107</sup>

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13 Awareness of the issues considered above, coupled with optimised dosing regimens  
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15 and plasma drug monitoring to maintain a therapeutic range between 0.15 -0.6mg / L  
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17 (approximately 0.5-2  $\mu\text{M}$ )<sup>108-110</sup>, have led to the re-introduction of PHX in the UK on a named  
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19 patient basis. However, phospholipidosis-linked toxicity (similar to that observed with chronic  
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21 amiodarone exposure)<sup>111</sup> raises the issue of accumulation in tissues which requires further  
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23 consideration of PHX from a physico-chemical perspective.

24  
25 PHX is very poorly water soluble (limit of solubility of 0.0608 mg / L; approximately  
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27 0.2  $\mu\text{M}$ ) and highly lipophilic- the logP, the octanol-water partition coefficient used as a  
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29 measure of lipophilicity is 6.2, comparable to that of amiodarone, a drug for which  
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31 membrane accumulation is a causal factor in its serious adverse effects (Table 2). Given the  
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33 very low water solubility of PHX, at safe clinical dosing levels (i.e. plasma concentration of  
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35 drug between 0.15 and 0.6 mg / L), the ratio of  $[\text{PHX}]_{\text{unbound}}$  to  $[\text{PHX}]_{\text{bound}}$  would be  
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37 approximately 40:60% and 10:90%, respectively. Importantly then, since under normal  
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39 (monitored) dosing conditions PHX would already be at its limit of solubility, any increase in  
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41 the concentration of PHX upwards of reportedly 'toxic' plasma levels of drug (i.e. > 0.6 mg /  
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43 L) would simply mean that more of the drug existed in a bound state in the plasma. The  
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45 notion therefore that monitoring plasma levels of PHX does not give a true indication of drug  
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47 distribution was recently confirmed by the remarkable finding of Drury and colleagues that  
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49 the concentrations of PHX in human right atria and left ventricles were 6.0 and 10.0 mg / kg,  
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51 respectively- approximately 100- to 170- times higher than the amount of PHX unbound  
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53 ('free') in the plasma.<sup>112</sup> The massive accumulation of drug was corroborated by the same  
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55 authors in a recent study.<sup>103</sup>

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3 So how does PHX accumulate to such high levels in tissue? We describe the  
4 possible mechanisms that underpin accumulation of the drug in section 6, but it is first  
5 necessary to consider the impact that drug ionization state will have on the modes of  
6 transfer across cellular membranes. The pKa of PHX is 10.58 so 99.9% of the drug would  
7 exist in the charged (cationic) form in the plasma at pH7.4. As discussed above then under  
8 therapeutic dosing regimens (i.e. at its limit of solubility of 0.06 mg / L), effectively all of the  
9 drug would exist in the cationic form ( $\approx 99.9\%$ , 0.05994 mg / L) with the neutral form present  
10 at just 0.00006 mg / L. If the tiny amount of the neutral form of the drug ( $\approx 60$  ng / L in the  
11 plasma) was the only species able to transfer across the cell surface membrane, myocardial  
12 concentrations of the drug at 6 – 10 mg /kg<sup>103, 112</sup> would establish an intracellular:plasma  
13 concentration gradient of 100,000:1. It is inconceivable that such massive accumulation  
14 could be supported by simple passive transfer of the neutral form of the drug from the  
15 plasma into cells, even if one considers that the neutral species that does partition across  
16 the surface membrane would be immediately protonated (to its cationic form) in the cellular  
17 environment (Figure 2). This therefore raises the likelihood that other factors contribute to  
18 the cellular accumulation of PHX. First, the peculiar hydrophobicity properties of PHX may  
19 promote the integration of the cationic species into the membranes. Second, and probably  
20 the most likely contributor to the cellular accumulation of PHX, is that organic ion transporter  
21 proteins (TP) present in the surface membrane are likely involved in the active uptake of the  
22 cationic form of the drug into cells, as has been reported to occur with amiodarone, a drug  
23 with a very similar physico-chemical profile to PHX.<sup>113</sup>

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48 **(6) Possible mechanisms of PHX accumulation in tissues.**

49 Some of the acute effects of PHX, at least in experimental systems, are rapidly reversible. In  
50 chick embryo ventricular cells, Barry *et al.* described the negative inotropic effect of PHX  
51 measured after a seven minute equilibration to reach steady state drug effects which was  
52 quickly reversed by washing out the drug.<sup>114</sup> However, as described in section 5, under  
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3 conditions of clinical dosing it appears that the therapeutic (and in some cases the  
4 hazardous) effects of PHX are primarily mediated by longer term accumulation inside cells.  
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7 Figure 2 describes some of the mechanisms that may contribute to PHX  
8 accumulation in the myocardium. Any cellular accumulation would be dependent on factors  
9 that disturb the free equilibrium between the cells and plasma and thus the only way that the  
10 drug can accumulate to levels grossly in excess of the plasma concentration is if there is an  
11 intracellular binding target or the relatively stable association of the drug with lipid  
12 membranes. Consequently, the scenario depicted in Figure 2A which describes the loading  
13 of cytoplasm with high  $[PHX]_{free}$ , and which may be commonly thought of as 'cytoplasmic  
14 accumulation', cannot occur. Also refuting the possibility that high cytoplasmic  $[PHX]_{free}$  (as  
15 depicted in Figure 2A) occurs *in situ* is that under these conditions the surface membrane  
16  $Na^+$ ,  $K^+$  and  $Ca^{2+}$  channels would also be exposed to high concentrations of drug. Since  
17 the mode of channel inhibition is through drug interaction at the cytoplasmic face of the  
18 channel and in consideration of the comparatively low  $IC_{50}$  values for PHX-channel  
19 interaction (Table 1) these channels would be profoundly inhibited. This would result in  
20 catastrophic impact on heart rhythm and rate, effects that are conspicuously absent in PHX  
21 treated patients (although a heart rate lowering effect of PHX in other species has been  
22 reported<sup>115, 116</sup>).

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39 Cellular accumulation could however feasibly occur via a mechanism that involves  
40 PHX binding to cytoplasmic moieties which would allow the drug to partition against its  
41 concentration gradient (Figure 2B). It is important to note though that in these  
42 circumstances, high levels of intracellular accumulation would not translate into high levels  
43 of drug concentration, and the levels of 'free' PHX in the cytoplasm would be low (Figure  
44 2B). It is possible though that low levels of 'free' PHX in the cytoplasm, which would  
45 predominantly be in the cationic form (see above), would still be sufficient to modulate  
46 surface membrane ion channel activity (Table 1).  
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56 There is evidence of preferential association of PHX with phospholipid membranes,  
57 particularly those characterized by regional 'charge' gradients (e.g.  $H^+$  gradients across  
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3 mitochondrial and lysosomal membranes)<sup>59, 71, 117</sup> which may contribute to intra-cellular  
4 localization of very high drug concentrations at these organelles. Such a mechanism could  
5 also conceivably lead to cellular ‘trapping’ of PHX. As to whether the protonation of PHX in  
6 the cytoplasm of the cell would lead to a buffering of H<sup>+</sup> ions and as a consequence  
7 modulate cellular pH, we believe this to be unlikely. In our view, any effect of PHX on pH  
8 normalization should remain focused on the relative balance of pyruvate-to-lactate which is  
9 dictated by the schemes outlined in Figure 1.  
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17 From its lipophilicity profile, the partitioning of PHX into membranes (especially  
18 mitochondrial membranes once it is protonated inside the cell) is likely to be rapid and it has  
19 been reported that high concentrations of drug applied extracellularly (25 µM) affected  
20 metabolism and promoted cellular toxicity within 24h.<sup>89</sup> Even more striking is the direct  
21 biochemical evidence for the decreased utilization of fatty acids (by approximately 35%) in  
22 rat hearts perfused with 2 µM PHX within a one-hour experimental window.<sup>59</sup> The timings of  
23 these effects point to either rapid cellular accumulation or alternatively, to an acute indirect  
24 modulation of metabolism (i.e. downstream from inhibition of surface-membrane ion  
25 channels).  
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35 There are two plausible scenarios in which longer-term accumulation of PHX could  
36 modulate CPT-1 and its other targets at the IC<sub>50</sub>s described in the literature (Table 1). First,  
37 if CPT-1 and the other targets **are** the target for PHX binding (Figure 2C). Under these  
38 conditions one would expect marked inhibition of surface membrane ion channel fluxes and,  
39 for the reasons outlined above, this is unlikely to occur *in vivo*. Specifically with regards  
40 CPT-1 though, the scheme described in Figure 2C may seem unlikely because of the  
41 endogenous competition from the much more potent malonyl CoA and the very high IC<sub>50</sub> of  
42 PHX (>100 µM in a *P. pastoris* expression system)<sup>49</sup> (Table 1). However, PHX interacts with  
43 CPT-1 via an atypical mechanism that possibly involves interaction within the plane of the  
44 membrane (see section 4, above) and thus could reach very high concentrations in the  
45 vicinity of CPT-1. Such massively localized accumulation of PHX at CPT-1 would constitute  
46 ‘active-site concentration’<sup>111</sup> although little is known as to how this phenomena may  
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3 influence drug-target inhibition. The idea that PHX preferentially partitions in the  
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5 extravascular pericardial compartment or pericardial fat (as occurs with amiodarone)<sup>49</sup> is not  
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7 supported by Drury's study which determined very high levels of PHX accumulation in  
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9 myocardial tissue obtained via biopsy of patients undergoing coronary artery bypass surgery  
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11 which was presumably free of pericardial fat.<sup>112</sup>  
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13 The second scenario for chronic PHX accumulation, and in our view the most likely  
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15 mechanism that reconciles the low potency of PHX for CPT-1 with its actions on this target  
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17 and on other ion channels is shown in Figure 2D. In this scheme PHX distribution is  
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19 heterogeneous and (1) it accumulates in the membrane, (2) it interacts directly with CPT-1  
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21 and (3) some of the drug is free in the cytoplasm as a result of an equilibrium set up  
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23 between cytoplasmic binding factor(s) and the bulk hydrophilic environment. All of these  
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25 distributions though are subject to saturability such that eventually all targets/binding  
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27 partners would be PHX bound or associated with intracellular moieties. After dosing  
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29 previously drug naïve patients at a plasma concentration of approximately 1  $\mu\text{M}$ ,  
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31 concentrations of PHX in ventricular tissue reached over 10 mg / kg (approximately 36  $\mu\text{M}$ )  
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33 within 30 days of drug administration (median 8.5 days).<sup>112</sup> Moreover, although the PHX  
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35 accumulation in these tissues was directly correlated with plasma concentration of drug and  
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37 length of therapy there was **no** evidence of saturability of uptake over the time of study.<sup>112</sup> In  
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39 a more recent follow-up, the conclusion of Chong and colleagues that PHX does exhibit  
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41 saturability is not supported by their data (see<sup>103</sup>, Fig 2A and B). Given these observations, it  
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43 is difficult to conceive of how long-term PHX administration, even with maintenance of  
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45 plasma concentrations of the drug at around 1  $\mu\text{M}$ , would not lead to the steady  
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47 accumulation of toxic levels of PHX. However, one of the authors of this article (ZY) has  
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49 been treating some HF patients with PHX for over ten years; this chronic administration of  
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51 PHX is well tolerated and the symptomatic improvements are substantial. Clearly, there are  
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53 factors that affect PHX tolerance and accumulation that remain incompletely understood.  
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56 Finally, one commonly overlooked feature of CPT-1 is its widespread tissue  
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58 distribution. CPT-1 is expressed at lower levels in the heart than in other tissues including  
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3 kidney, liver, pancreas, lung and intestine.<sup>49</sup> The issues considered above would presumably  
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5 apply equally in these tissues and the reported patterns of hepato-, neuro- and gastrotoxicity  
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7 are consistent with this idea.  
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### 10 11 **(7) Expert Opinion.** 12

13 In this article we have considered issues relating to the chemistry, mechanism of action,  
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15 tissue accumulation and clinical benefits of PHX. An improved understanding of PHX drug  
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17 safety and monitoring has underpinned its use in patients with HF and the evidence for its  
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19 therapeutic efficacy in this context is strong. However, the disparate clinical endpoints  
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21 following PHX administration in HF arising from distinct aetiologies (e.g. ischaemic- versus  
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23 non-ischaemic HF) suggest that the mechanism of action of PHX is complex and not fully  
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25 understood.  
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27 The more recent literature is dominated by the concept that the beneficial effects of  
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29 PHX are due primarily to its inhibition of myocardial CPT-1. Contrary to this dogma, having  
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31 reviewed all the available evidence, including key patents relating to the clinical usage of  
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33 PHX and detailed studies of drug-target interactions, we suggest that it is very unlikely that  
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35 the *major* effects of PHX are due to CPT-1 inhibition. Furthermore, over the last twenty years  
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37 or so the preoccupation with CPT-1 as the most relevant target of PHX has overshadowed a  
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39 substantive body of older, but very credible evidence, for PHX affecting the extra-cardiac  
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41 circulatory system. It is entirely likely that the therapeutic benefits of PHX in HF are due to  
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43 combined cardiac and vascular effects (e.g. vasodilation).  
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45 The robustness of some of the statements made regarding PHX and CPT-1 inhibition  
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47 is at stark odds with the limited evidence supporting such a mechanism that can be found in  
48  
49 the literature. In a recent and detailed analysis of CPT-1 inhibitor chemistry, Ceccarelli  
50  
51 effectively debunked the idea that perhexiline is a physiologically relevant CPT-1 inhibitor.<sup>49</sup>  
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53 However, we cannot completely exclude the possibility that **minor** effects of PHX on CPT-1  
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55 underpin disproportionately large effects on myocardial function, especially since there is  
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57 massive accumulation of the drug in atrial and ventricular tissue. To reconcile these  
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3 arguments, it is plausible that the symptomatic improvements determined in HF patients are  
4 due to the fact that PHX is **not** a good CPT-1 inhibitor in an *in vivo* setting and that any  
5 clinical utility is dependent on only partial CPT-1 inhibition. Horowitz and colleagues, who  
6 first positioned PHX as an exemplar CPT-1 inhibitor<sup>60, 71</sup>, have previously acknowledged this  
7 likelihood, albeit from the perspective of toxicity rather than efficacy- “the adverse reactions  
8 encountered with perhexiline and indeed those of other CPT-1 inhibitors (e.g. etomoxir) are  
9 the consequence of excessive CPT-1 and hence fatty acid metabolism inhibition”.<sup>71</sup> Like  
10 PHX, the antianginal trimetazidine exerts multiple effects via multiple mechanisms (see  
11 e.g.<sup>118</sup>); it is a potent inhibitor of beta-oxidation of free fatty acids<sup>119</sup>, but only a weak CPT1  
12 inhibitor<sup>120</sup>, and has been shown to reduce fibrosis<sup>121</sup> and to normalise aberrant Ca<sup>2+</sup>  
13 handling in cardiomyocytes obtained from post-injured myocardium.<sup>122</sup> Moreover, the finding  
14 that etomoxir, the only drug considered as a *bona fide* CPT-1 inhibitor by Ceccarelli and  
15 colleagues<sup>49</sup> evoked severe hepatotoxicity<sup>123</sup> would also suggest that appreciable inhibition of  
16 CPT-1 *in vivo* is not clinically useful. Thus although there is undeniable merit in developing  
17 new ‘metabolic modulators’, approaches that aim to develop selective CPT-1 targeted drugs  
18 with enhanced biological half-life and/or increased potency should be viewed with caution. It  
19 is also likely that targets other than CPT-1 present alternative strategies for regulating  
20 myocardial FA fluxes.<sup>49, 124</sup>

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The linked nature of the composite systems involved in metabolic regulation (i.e. the complex synchronization of ion handling, metabolite sensing, substrate utilization, energy supply-and-demand) means that reconciling any effects on altered energy metabolism with the specific effects on discrete molecular targets is extremely difficult. With this in mind, the evidence that PHX effects are mediated by its actions on a multiple ion channels and other cellular components that impinge on aspects of cellular metabolism is more convincing (Table 1). In Figure 2D, we propose a scheme which considers the effects of PHX via 1) the inhibition of surface membrane ion channels via low levels of free drug in the cytoplasm and 2) accumulation in mitochondrial membranes which leads to a highly localized concentration of drug in the vicinity of CPT-1 resulting in its partial inhibition.

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3 In summary, we suggest that due to its physico-chemical properties, PHX is a drug of  
4 low potency, low specificity and low selectivity. As a result, the pleiotropic actions of PHX on  
5 a diverse range of molecular targets, some of which remain to be properly defined,  
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7 contribute to its therapeutic benefit in HF.  
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**Conflict of interest**

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## References

Reference annotations

\* of interest

\*\* of considerable interest

1. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA Guideline for the Management of Heart Failure: A report of the American College of Cardiology Foundation / American Heart Association Task Force on practice guidelines. *Circulation* 2013;128:e240-e327.
2. Tiku Owens A, Brozena SC, Jessup M. New managements strategies in heart failure. *Circ Res* 2016;118:480-95.
3. Braunwald E. Research advances in heart failure: a compendium. *Circ Res* 2013;113:633-45.
4. George CH, Barberini-Jammaers SR, Muller CT. Refocussing therapeutic strategies for cardiac arrhythmias: defining viable molecular targets to restore cardiac ion flux. *Expert Opin Ther Patents* 2008;18:1-19.
5. von Leuder TG, Krum H. New medical therapies for heart failure. *Nat Rev Cardiol* 2015;12:730-40.
6. Sanganalmath SK, Bolli R. Cell therapy for heart failure: a comprehensive overview of experimental and clinical studies, current challenges, and future directions. *Circ Res* 2013;113:810-34.
7. George CH. Sarcoplasmic reticulum  $Ca^{2+}$  leak in heart failure: mere observation or functional relevance? *Cardiovasc Res* 2008;77:302-14.
8. George CH, Parthimos D, Silvester NC. A network-oriented perspective on cardiac calcium signaling. *Am J Physiol Cell Physiol* 2012;303:C897-C910.
9. Boileau E, George CH, Parthimos D, et al. Synergy between intercellular communication and intracellular  $Ca^{2+}$  handling in arrhythmogenesis. *Ann Biomed Engineer* 2015;43:1614-25.

10. Antoons G, Sipido KR. Targeting calcium handling in arrhythmias. *Europace* 2008;10:1364-69.
11. Bers DM, Harris SP. To the rescue of the failing heart. *Nature* 2011;473:36-39.
12. Anderson ME. Multiple downstream proarrhythmic targets for calmodulin kinase II: moving beyond an ion channel-centric focus. *Cardiovasc Res* 2007;73:657-66.
13. Wehrens XHT, Marks AR. Novel therapeutic approaches for heart failure by normalizing calcium cycling. *Nat Rev Drug Disc* 2004;3:1-9.
14. Yano M, Yamamoto T, Ikeda Y, et al. Mechanisms of Disease: ryanodine receptor defects in heart failure and fatal arrhythmia. *Nat Clin Pract Cardiovasc Med* 2006;3:43-52.
- \*\*15. Ashrafian H, Horowitz JD, Frenneaux MP. Perhexiline. *Cardiovasc Drug Rev* 2007;25:76-97.  
*A key review article on the history of PHX*
16. Inglis S, Stewart S. Metabolic therapeutics in angina pectoris: history revisited with perhexiline. *Eur J Cardiovasc Nurs* 2006;5:176-84.
17. Lee L, Horowitz J, Frenneaux M. Metabolic manipulation in ischaemic heart disease, a novel approach to treatment. *Eur Heart J* 2004;25:634-41.
18. Singh S, Schwarz K, Horowitz J, et al. Cardiac energetic impairment in heart disease and the potential role of metabolic modulators: a review for clinicians. *Circ Cardiovasc Genet* 2014;7:720-28.
19. Chong CR, Sullivan DJ. New uses for old drugs. *Nature* 2007;448:645-46.
20. Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. *Nat Rev Drug Discov* 2004;3:673-83.
21. Keiser MJ, Setola V, Irwin JJ, et al. Predicting new molecular targets for known drugs. *Nature* 2009;462:175-81.
22. Silvester NC, George CH. Searching for new cardiovascular drugs: towards improved systems for drug screening? *Expert Opin Drug Discov* 2011;6:1155-70.



- 1  
2  
3 23. Lewis KJ, Silvester NC, Barberini-Jammaers SR, et al. A new system for profiling  
4 drug-induced calcium signal perturbation in human embryonic stem cell-derived  
5 cardiomyocytes. *J Biomol Screen* 2015;20:330-40.  
6  
7  
8  
9 24. Fleckenstein A. Specific pharmacology of calcium in myocardium, cardiac  
10 pacemakers, and vascular smooth muscle. *Ann Rev Pharmacol Toxicol* 1977;17:149-66.  
11  
12 \*\*25. Vaughan Williams EM. *Antiarrhythmic action and the puzzle of perhexiline*. London:  
13 Academic Press, 1980.  
14  
15 *A valuable resource that documents many of the historical 'puzzles' that the present review*  
16 *attempts to resolve given our contemporary knowledge of PHX*  
17  
18  
19  
20  
21 26. Fleckenstein A. History of calcium antagonists. *Circ Res* 1983;52 (Suppl. I):3-16.  
22  
23 27. Spedding M. Changing surface charge with salicylate differentiates between  
24 subgroups of calcium-antagonists. *Br J Pharmacol* 1984;83:211-20.  
25  
26  
27 28. Barry WH, Horowitz JD, Smith TW. Comparison of negative inotropic potency,  
28 reversibility, and effects on calcium influx of six calcium channel antagonists in cultured  
29 myocardial cells. *Br J Pharmacol* 1985;85:51-59.  
30  
31  
32 29. Cole PL, Beamer AD, McGowan N, et al. Efficacy and safety of perhexiline maleate  
33 in refractory angina. A double-blind placebo-controlled clinical trial of a novel antianginal  
34 agent. *Circulation* 1990;81:1260-70.  
35  
36  
37 30. Bleifer DJ, Bleifer SB, Okun R. Perhexiline maleate in angina pectoris: a controlled,  
38 double-blind clinical trial. *Geriatrics* 1972;27:109-15.  
39  
40  
41 31. Burns-Cox CJ, Chandrasekhar KP, Ikram H, et al. Clinical evaluation of perhexiline  
42 maleate in patients with angina pectoris. *B M J* 1971;4:586-88.  
43  
44  
45 32. Liberts EA, Willoughby SR, Kennedy JA, et al. Effects of perhexiline and nitroglycerin  
46 on vascular, neutrophil and platelet function in patients with stable angina pectoris. *Eur J*  
47 *Pharmacol* 2007;560:49-55.  
48  
49  
50 33. Lyon LJ, Nevin MA, Fisch S, et al. Perhexiline maleate in treatment of angina  
51 pectoris. *Lancet* 1971;June:1272-74.  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 34. White HD, Lowe JB. Antianginal efficacy of perhexiline maleate in patients refractory  
4 to beta-adrenoceptor blockade. *Int J Cardiol* 1983;3:145-55.  
5  
6  
7 35. Willoughby SR, Stewart S, Chirkov YY, et al. Beneficial clinical effects of perhexiline  
8 in patients with stable angina pectoris and acute coronary syndromes are associated with  
9 potentiation of platelet responsiveness to nitric oxide. *Eur Heart J* 2002;23:1946-54.  
10  
11  
12 36. Ashrafian H, Frenneaux M, Opie LH. Metabolic mechanisms in heart failure.  
13 *Circulation* 2007;116:434-48.  
14  
15  
16  
17 37. Ingwall JS. Energy metabolism in heart failure and remodelling. *Cardiovasc Res*  
18 2009;81:412-19.  
19  
20  
21 38. Neubauer S. Mechanisms of disease: the failing heart- an engine out of fuel. *N Engl*  
22 *J Med* 2007;356:1140-51.  
23  
24  
25 39. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the  
26 normal and failing heart. *Physiol Rev* 2005;85:1093-129.  
27  
28  
29 40. Tuunanen H, Knuuti J. Metabolic remodelling in human heart failure. *Cardiovasc Res*  
30 2011;90:251-57.  
31  
32  
33 41. Ventura-Clapier R, Garnier A, Veksler V. Energy metabolism in heart failure. *J*  
34 *Physiol* 2004;555:1-13.  
35  
36  
37 42. Doenst T, Nguyen TD, Abel ED. Cardiac metabolism in heart failure: implications  
38 beyond ATP production. *Circ Res* 2013;113:709-24.  
39  
40  
41 43. Dass S, Holloway CJ, Cochlin LE, et al. No evidence of myocardial oxygen  
42 deprivation in nonischemic heart failure. *Circ Heart Fail* 2015;8:1088-93.  
43  
44  
45 44. Stanley WC, Chandler MP. Energy metabolism in the normal and failing heart:  
46 potential for therapeutic interventions. *Heart Fail Rev* 2002;7:115-30.  
47  
48  
49 45. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control.  
50 *Nat Rev Mol Cell Biol* 2009;10:307-18.  
51  
52  
53 46. Arad M, Seidman CE, Seidman JG. AMP-activated protein kinase in the heart: role  
54 during health and disease. *Circ Res* 2007;100:474-88.  
55  
56  
57  
58  
59  
60

- 1  
2  
3 47. Mihaylova MM, Shaw RJ. The AMPK signalling pathway coordinates cell growth,  
4 autophagy and metabolism. *Nat Cell Biol* 2011;13:1016-23.  
5  
6  
7 48. Shimobayashi M, Hall MN. Making new contacts: the mTOR network in metabolism  
8 and signalling crosstalk. *Nat Rev Mol Cell Biol* 2014;15:155-62.  
9  
10  
11 \*\*49. Ceccarelli SM, Chomienne O, Gubler M, et al. Carnitine palmitoyltransferase (CPT)  
12 modulators: a medicinal chemistry perspective on 35 years of research. *J Med Chem*  
13 2011;54:3109-52.  
14  
15  
16  
17 *An outstanding and in-depth review of the medicinal chemistry of CPT-1 modulators.*  
18  
19 50. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system:  
20 from concept to molecular analysis. *Eur J Biochem* 1997;244:1-14.  
21  
22  
23 51. Chugai Seiyaku Kabushiki Kaisha. Heterocyclic compounds useful as malonyl-CoA  
24 decarboxylase inhibitors. US7,696,365. 2010.  
25  
26  
27 52. Chugai Seiyaku Kabushiki Kaisha. Piperidine compounds useful as malonyl-CoA  
28 decarboxylase inhibitors. US7,449,482. 2008.  
29  
30  
31 53. Dyck JRB, Lopaschuk GD. Malonyl CoA control of fatty acid oxidation in the ischemic  
32 heart. *J Mol Cell Cardiol* 2002;34:1099-109.  
33  
34  
35  
36 \*\*54. Lee L, Campbell R, Scheuermann-Freestone M, et al. Metabolic modulation with  
37 perhexiline in chronic heart failure: a randomized, controlled trial of short-term use of a novel  
38 treatment. *Circulation* 2005;112:3280-88.  
39  
40  
41 *A seminal publication describing the symptomatic benefit of PHX treatment in heart failure*  
42  
43 55. Abozguia K, Elliott P, McKenna W, et al. Metabolic modulator perhexiline corrects  
44 energy deficiency and improves exercise capacity in symptomatic hypertrophic  
45 cardiomyopathy. *Circulation* 2010;122:1562-69.  
46  
47  
48  
49 56. Beadle RM, Williams LK, Kuehl M, et al. Improvement in cardiac energetics by  
50 perhexiline in heart failure due to dilated cardiomyopathy. *JACC: Heart Failure* 2015;3:202-  
51 11.  
52  
53  
54  
55 57. Lee ESP, Edmunds L, Fraser AG, et al. Perhexiline improves functional outcome in  
56 patients with refractory angina and heart failure. *Eur J Heart Fail* 2013;12:S241.  
57  
58  
59  
60

- 1  
2  
3 58. Pepine CJ, Schang SJ, Bemiller CR. Effects of perhexiline on coronary  
4 hemodynamic and myocardial metabolic responses to tachycardia. *Circulation*  
5 1974;XLIX:887-93.  
6  
7  
8  
9 59. Jeffery FMH, Alvarez L, Diczku V, et al. Direct evidence that perhexiline modifies  
10 myocardial substrate utilization from fatty acids to lactate. *J Cardiovasc Pharmacol*  
11 1995;25:469-72.  
12  
13  
14 \*60. Queen Elizabeth Hospital. Methods related to the treatment of and isolation of  
15 compounds for treatment of ischaemic conditions. WO1997000678. 1997.  
16  
17 *Patent describing the use of an assay based on CPT-1 inhibition*  
18  
19  
20  
21 61. Heart Metabolics Limited. Perhexiline for treating chronic heart failure. US8,470,806.  
22 2013.  
23  
24  
25 62. Heart Metabolics Limited. Treatment of heart failure. US8,440,697. 2013.  
26  
27  
28 63. University of Birmingham. Perhexiline for use in the treatment of hypertrophic  
29 cardiomyopathy (HCM). US8,697,728. 2014.  
30  
31  
32 64. University Court of the University of Aberdeen. Fluoro-perhexiline compounds and  
33 their therapeutic use. WO2014184561. 2014.  
34  
35  
36 65. Chirkov YY, Holmes AS, Willoughby SR, et al. Stable angina and acute coronary  
37 syndromes are associated with nitric oxide resistance in platelets. *J Am Coll Cardiol*  
38 2001;37:1851-57.  
39  
40  
41 66. Hudak WJ, Lewis RE, Kuhn WL. Cardiovascular pharmacology of perhexiline. *J*  
42 *Pharm Exp Ther* 1970;137:371-82.  
43  
44  
45 67. O'Hara N, Ono H, Ognuro K, et al. Vasodilating effects of perhexiline, glyceryl  
46 trinitrate, and verapamil on the coronary, femoral, renal, and mesenteric vasculature of the  
47 dog. *J Cardiovasc Pharmacol* 1981;3:251-68.  
48  
49  
50  
51 68. Morano I, Isac M, Bletz C, et al. Perhexiline increases calcium-activated force in  
52 skinned psoas fibres by raising calcium affinity of troponin-C. *Biomed Biochim Acta*  
53 1989;48:S329-S34.  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 69. Kennedy JA, Beck-Oldach K, McFadden-Lewis K, et al. Effect of the anti-anginal  
4 agent, perhexiline, on neutrophil, valvular and vascular superoxide formation. Eur J  
5 Pharmacol 2006;531:13-19.  
6  
7  
8  
9 70. Frenneaux M. New tricks for an old drug. Eur Heart J 2002;23:1898-99.  
10  
11 \*\*71. Kennedy JA, Unger SA, Horowitz J. Inhibition of carnitine palmitoyltransferase-1 in  
12 rat heart and liver by perhexiline and amiodarone. Biochem Pharmacol 1996;52:273-80.  
13  
14 *The original paper in which PHX was proposed as a CPT-1 inhibitor*  
15  
16  
17 72. Jackson VN, Cameron JM, Fraser F, et al. Use of six chimeric proteins to investigate  
18 the role of intramolecular interactions in determining the kinetics of carnitine  
19 palmitoyltransferase I isoforms. J Biol Chem 2000;275:19560-66.  
20  
21  
22  
23 73. Jackson VN, Cameron JM, Zammit VA, et al. Sequencing and functional expression  
24 of the malonyl-CoA-sensitive carnitine palmitoyltransferase from Drosophila melanogaster.  
25  
26 Biochem J 1999;341:483-89.  
27  
28  
29 74. Heygi B, Komaromi I, Nanasi PP, et al. Selectivity problems with drugs acting on  
30 cardiac Na<sup>+</sup> and Ca<sup>2+</sup> channels. Curr Med Chem 2013;20:2552-71.  
31  
32  
33  
34 75. George CH, Thomas NL, Lai FA. Ryanodine receptor dysfunction in arrhythmia and  
35 sudden cardiac death. Future Cardiol 2005;1:531-41.  
36  
37  
38 \*76. Mencher SK, Wang LG. Promiscuous drugs compared to selective drugs  
39 (promiscuity can be a virtue). BMC Clin Pharmacol 2005;5:3.  
40  
41 *A thought-provoking opinion on drug pleiotropy*  
42  
43  
44 77. Smith BK, Perry CGR, Koves TR, et al. Identification of a novel malonyl-CoA IC<sub>50</sub> for  
45 CPT-1: implications for predicting in vivo fatty acid oxidation rates. Biochem J 2012;448:13-  
46  
47 20.  
48  
49  
50 78. Stephens TW, Higgins AJ, Cook GA, et al. Two mechanisms produce tissue-specific  
51 inhibition of fatty oxidation by oxfenicine. Biochem J 1985;227:651-60.  
52  
53  
54 79. Kennedy JA, Kiosoglous AJ, Murphy GA, et al. Effect of perhexiline and oxfenicine  
55 on myocardial function and metabolism during low-flow ischemia/reperfusion in the isolated  
56 rat heart. J Cardiovasc Pharmacol 2000;36:794-801.  
57  
58  
59  
60

- 1  
2  
3 \*80. Patel A, Jones SA, Ferro A, et al. Pharmaceutical salts: a formulation trick or a  
4 clinical conundrum. *Br J Cardiol* 2009;16:281-86.  
5  
6  
7 *An interesting perspective on the physiological effects of counterions in pharmaceutical*  
8 *formulations*  
9  
10  
11 81. Pacanis A, Strzelecki T, Rogulski J. Effects of maleate on the content of CoA and its  
12 derivatives in rat kidney mitochondria. *J Biol Chem* 1981;256:13035-38.  
13  
14 82. Rogulski J, Pacanis A, Strzelecki T, et al. Effects of maleate on carbohydrate  
15 metabolism in rats. *Am J Physiol* 1976;230:1163-67.  
16  
17  
18 83. Tuncel AT, Ruppert T, Wang B-T, et al. Maleic acid- but not structurally related  
19 methylmalonic acid- interrupts energy metabolism by impaired calcium homeostasis. *PLoS*  
20 *One* 2015;10:e0128770.  
21  
22  
23 84. Yin X, Dwyer J, Langley SR, et al. Effects of perhexiline-induced fuel switch on the  
24 cardiac proteome and metabolome. *J Mol Cell Cardiol* 2013;55:27-30.  
25  
26  
27 85. Gehmlich K, Dodd MS, Allwood JW, et al. Changes in the cardiac metabolome  
28 caused by perhexiline treatment in a mouse model of hypertrophic cardiomyopathy. *Mol*  
29 *Biosyst* 2015;11:564.  
30  
31  
32 86. Gatto Jr GJ, Ao Z, Kearse MG, et al. NADPH oxidase-dependent and -independent  
33 mechanisms of reported inhibitors of reactive oxygen generation. *J Enzyme Inhib Med Chem*  
34 2013;28:95-104.  
35  
36  
37 87. Unger SA, Kennedy JA, McFadden-Lewis K, et al. Dissociation between metabolic  
38 and efficiency effects of perhexiline in normoxic rat myocardium. *J Cardiovasc Pharmacol*  
39 2005;46:849-55.  
40  
41  
42 88. Shah RR, Oates NS, Idle JR, et al. Impaired oxidation of debrisoquine in patients  
43 with perhexiline neuropathy. *BMJ* 1982;284:295-99.  
44  
45  
46 89. Deschamps D, DeBeco V, Fisch C, et al. Inhibition by perhexiliene of oxidative  
47 phosphorylation and the B-oxidation of fatty acids: possible role in pseudoalcoholic liver  
48 lesions. *Hepatology* 1994;19:948-61.  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1  
2  
3 90. Shah RR. Can pharmacogenetics help rescue drugs withdrawn from market?  
4  
5 Pharmacogenomics 2006;7:889-908.  
6  
7 91. Singlas E, Goujet MA, Simon P. Pharmacokinetics of perhexiline maleate in anginal  
8  
9 patients with and without peripheral neuropathy. *Europ J Clin Pharmacol* 1978;14:195-201.  
10  
11 92. Afzal Mir M, Kafetzakis EM. Assessment of perhexiline maleate in angiographically  
12  
13 proven intractable angina: a double-blind trial. *Am Heart J* 1978;96:350-54.  
14  
15 93. Morgan MY, Reshef R, Shah RR, et al. Impaired oxidation of debrisoquine in patients  
16  
17 with perhexiline liver injury. *Gut* 1984;25:1057-64.  
18  
19 94. Fromenty B, Pessayre D. Inhibition of mitochondrial beta-oxidation as a mechanism  
20  
21 of hepatotoxicity. *Pharmac Ther* 1995;67:101-54.  
22  
23 95. Cooper RG, Evans DAP, Price AH. Studies on the metabolism of perhexiline in man.  
24  
25 *Eur J Clin Pharmacol* 1987;32:569-76.  
26  
27 96. Gonzales FJ, Skoda RC, Kimura S, et al. Characterization of the common genetic  
28  
29 defect in humans deficient in debrisoquine metabolism. *Nature* 1988;331:442-46.  
30  
31 97. Sallustio BC, Westley IS, Morris RG. Pharmacokinetics of the antianginal agent  
32  
33 perhexiline: relationship between metabolic ratio and steady-state dose. *Br J Clin Pharmacol*  
34  
35 2002;54:107-14.  
36  
37 98. Gardiner SJ, Begg EJ. Pharmacogenetics, drug-metabolizing enzymes, and clinical  
38  
39 practice. *Pharm Rev* 2006;58:521-90.  
40  
41 99. Amoah AG, Gould BJ, Parke DV. Single-dose pharmacokinetics of perhexiline  
42  
43 administered orally to humans. *J Chromatogr* 1984;305:401-09.  
44  
45 100. Amoah AG, Gould BJ, Parke DV, et al. Further studies on the pharmacokinetics of  
46  
47 perhexiline maleate in humans. *Xenobiotica; the fate of foreign compounds in biological*  
48  
49 *systems* 1986;16:63-68.  
50  
51 101. Cooper RG, Evans DAP, Whibley EJ. Polymorphic hydroxylation of perhexiline  
52  
53 maleate in man. *J Med Genet* 1984;21:27-33.  
54  
55 102. Hutt AJ, Tan SC. Drug chirality and its clinical significance. *Drugs* 1996;52 Suppl.  
56  
57 5:1-12.  
58  
59  
60

- 1  
2  
3 103. Chong CR, Drury NE, Licari G, et al. Stereoselective handling of perhexiline:  
4 implications regarding accumulation within the human myocardium. Eur J Clin Pharmacol  
5 2015;71:1485-91.  
6  
7  
8 104. Gould BJ, Amoah AG, Parke DV. Stereoselective pharmacokinetics of perhexiline.  
9 Xenobiotica 1986;16:491-502.  
10  
11 105. Inglis SC, Herbert MK, Davies BJ, et al. Effect of CYP2D6 metabolizer status on the  
12 disposition of the (+) and (-) enantiomers of perhexiline in patients with myocardial  
13 ischaemia. Pharmacogenet Genomics 2007;17:305-12.  
14  
15 106. Licari G, Sallustio BC, Somogyi AA, et al. The enantiomers of the myocardial  
16 metabolic agent perhexiline display divergent effects on hepatic energy metabolism and  
17 peripheral neural function in rats. Global Heart 2014;9:e272.  
18  
19 107. Adelaide Research & Innovation Pty Ltd; Central Adelaide Local Health Network Inc.;  
20 Itek Ventures Pty Ltd. Uses of (-)-perhexiline. WO2014036603. 2014.  
21  
22 108. Horowitz JD, Morris PM, Drummer OH, et al. High-performance liquid  
23 chromatography assay of perhexiline maleate in plasma. J Pharmacol Sci 1981;70:320-22.  
24  
25 109. Horowitz JD, Sia STB, Macdonald PS, et al. Perhexiline maleate treatment for severe  
26 angina pectoris- correlations with pharmacokinetics. Int J Cardiol 1986;13:219-29.  
27  
28 110. Stewart S, Voss DW, Northey DL, et al. Relationship between plasma perhexiline  
29 concentration and symptomatic status during short-term perhexiline therapy. Ther Drug  
30 Monit 1996;18:635-39.  
31  
32 111. Tylutki Z, Polak S. Plasma vs. heart tissue concentration in humans - literature data  
33 analysis of drugs distribution. Biopharm Drug Dispos 2015;36:337-51.  
34  
35 \*\*112. Drury NE, Licari G, Chong C-R, et al. Relationship between plasma, atrial and  
36 ventricular perhexiline concentrations in humans: insights into factors affecting myocardial  
37 uptake. Br J Clin Pharmacol 2013;77:789-95.  
38  
39 *A paper describing the remarkable levels of PHX that accumulate in cardiac tissue.*  
40  
41  
42  
43  
44  
45  
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48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60



- 1  
2  
3 113. Seki S, Kobayashi M, Itagaki S, et al. Contribution of organic anion transporting  
4 polypeptide OAT2B1 to amiodarone accumulation in lung epithelial cells. *Biochim Biophys*  
5 *Acta* 2009;1788:911-17.  
6  
7  
8  
9 114. Barry WH, Horowitz JD, Smith TW. Comparison of negative inotropic potency,  
10 reversibility, and effects on calcium influx of six calcium channel antagonists in cultured  
11 myocardial cells. *Br J Pharmacol* 1985;85:51-59.  
12  
13 115. Matsuo S, Cho YW, Aviado DM. Pharmacology of a new antianginal drug:  
14 perhexiline. II. Heart rate and transmembrane potential of cardiac tissue. *Chest*  
15 1970;58:581-85.  
16  
17  
18  
19 116. Yokoyama S, Konishi T, Matsuyama E, et al. Effects of perhexiline maleate on the  
20 refractory periods of isolated atrial muscle and atrioventricular node of the rabbit. *J*  
21 *Cardiovasc Pharmacol* 1982;4:26-31.  
22  
23  
24  
25 117. Shacoori V, Leray G, Guenet L, et al. Inhibition of (Na<sup>+</sup>,K<sup>+</sup>)-ATPase and Mg<sup>++</sup>-  
26 ATPase by a lysosomotropic drug: perhexiline maleate. *Res Commun Chem Pathol*  
27 *Pharmacol* 1988;59:161-72.  
28  
29  
30  
31 118. Chrusciel P, Rysz J, Banach M. Defining the role of trimetazidine in the treatment of  
32 cardiovascular disorders: some insights on its role in heart failure and peripheral artery  
33 disease. *Drugs* 2014;74:971-80.  
34  
35  
36  
37 119. Kantor PF, Lucien A, Kozak R, et al. The antianginal drug trimetazidine shifts cardiac  
38 energy metabolism from fatty acid oxidation to glucose oxidation by inhibiting mitochondrial  
39 long-chain 3-ketoacyl coenzyme A thiolase. *Circ Res* 2000;86:580-88.  
40  
41  
42  
43 120. Kennedy JA, Horowitz JD. Effect of trimetazidine on carnitine palmitoyltransferase-1  
44 in the rat heart. *Cardiovasc Drugs Ther* 1998;12:359-63.  
45  
46  
47  
48 121. Liu X, Gai Y, Liu F, et al. Trimetazidine inhibits pressure overload-induced cardiac  
49 fibrosis through NADPH oxidase-ROS-CTGF pathway. *Cardiovasc Res* 2010;88:150-58.  
50  
51  
52  
53 122. Meng D, Feng L, Chen X-J, et al. Trimetazidine improved Ca<sup>2+</sup> handling in  
54 isoprenaline-mediated myocardial injury of rats. *Exp Physiol* 2006;91:591-601.  
55  
56  
57  
58  
59  
60

- 1  
2  
3 \*123. Holubarsch CJF, Rohrbach M, Karrasch M, et al. A double-blind randomized  
4  
5 multicentre clinical trial to evaluate the efficacy and safety of low doses of etomoxir in  
6  
7 comparison with placebo in patients with moderate congestive heart failure: the ERGO  
8  
9 (etomoxir for the recovery of glucose oxidation) study. Clin Sci 2007;113:205-12.  
10  
11 *Paper giving foundation to the concept that CPT-1 inhibitors with improved*  
12  
13 *specificity/selectivity may not be clinically useful*  
14  
15 124. Luiken JJFP, Niessen HEC, Coort SLM, et al. Etomoxir-induced partial carnitine  
16  
17 palmitoyltransferase 1 (CPT-1) inhibition in vivo does not alter cardiac long-chain fatty acid  
18  
19 uptake and oxidation rates. Biochem J 2009;419:447-55.  
20  
21 125. Rampe D, Wang Z, Fermini B, et al. Voltage- and time-dependent block by  
22  
23 perhexiline of K<sup>+</sup> currents in human atrium and in cells expressing a Kv1.5-type cloned  
24  
25 channel. J Pharmacol Exp Ther 1995;274:444-49.  
26  
27 126. Perrin MJ, Kuchel PW, Campbell TJ, et al. Drug binding to the inactivated state is  
28  
29 necessary but not sufficient for high-affinity binding to human ether-a-go-go-related gene  
30  
31 channels. Mol Pharmacol 2008;74:1443-52.  
32  
33 127. Walker BD, Valenzuela SM, Singleton CB, et al. Inhibition of HERG channels stably  
34  
35 expressed in a mammalian cell line by the antianginal agent perhexiline maleate. Br J  
36  
37 Pharmacol 1999;127:243-51.  
38  
39 128. Grima M, Velly J, Decker N, et al. Inhibitory effects of some cyclohexylalkylamines  
40  
41 related to perhexiline on sodium influx, binding of [<sup>3</sup>H]batrachotoxinin A 20-alpha-benzoate  
42  
43 and [<sup>3</sup>H]nitrendipine and on guinea pig left atria contractions. Eur J Pharmacol  
44  
45 1988;147:173-85.  
46  
47 129. Kluppel MLW, Vieira Lopes LC, Silveira O, et al. Possible mechanism of action of  
48  
49 perhexiline maleate on heart mitochondria. Biochem Pharmacol 1976;25:2383-86.  
50  
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Rank	Target	IC <sub>50</sub>	Experimental system	Ref.
order of		( $\mu\text{M}$ )		
potency				

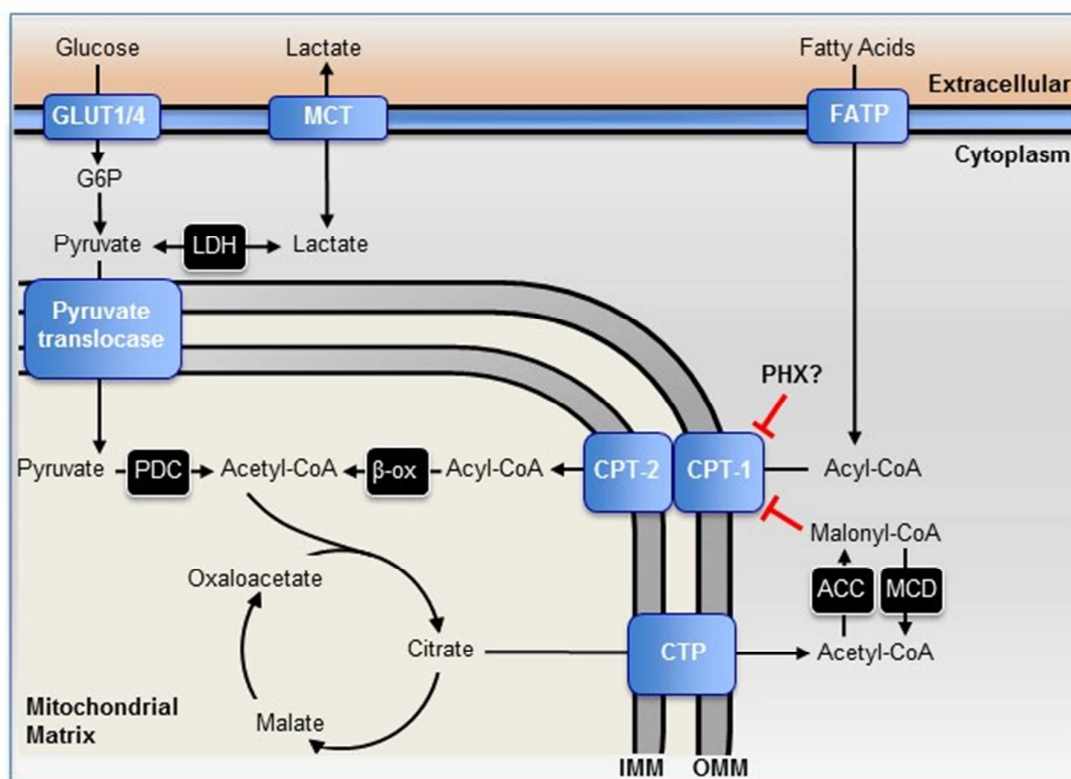
Table 1. Rank order of PHX inhibitory potency.

1	K <sub>v</sub> 1.5 (IK <sub>ur</sub> )	2	Recombinant Kv1.5 in HEK cells	125
2	NADPH oxidase	2 - 6	Neutrophils, cardiac fibroblasts	69, 86
3	HERG	6 - 8	Recombinant HERG in CHO cells	126, 127
4	'Fast' Na <sup>+</sup> channels	10 - 30	Rat heart membrane / synaptosomes	128
5	Ca <sup>2+</sup> channels	1 - 50	Rat heart membrane	128
6	NADH-CoQ (Complex I)	23 - 68	Rat heart mitochondria	129
7	Na <sup>+</sup> /K <sup>+</sup> -ATPase	50	Rat brain homogenate	117
8	CPT-2	73 - 79	Recombinant <i>Pichia pastoris</i> membrane / rat heart mitochondria	49, 79
9	CPT-1	77 - >100	Rat heart mitochondria / recombinant <i>Pichia pastoris</i> membrane	49, 79

K<sub>v</sub>1.5, K<sup>+</sup> channel carrying the ultra-rapid delayed rectifier current (IK<sub>ur</sub>); HEK, human embryonic kidney; CHO, Chinese hamster ovary; HERG, K<sup>+</sup> channel encoded by the human ether-a-go-go related gene that carries the rapid delayed rectifier current (IK<sub>r</sub>).

**Table 2. Chemical names, structures and logP values of Ca<sup>2+</sup>-, Na<sup>+</sup>-, K<sup>+</sup>-channel and CPT inhibitors.**

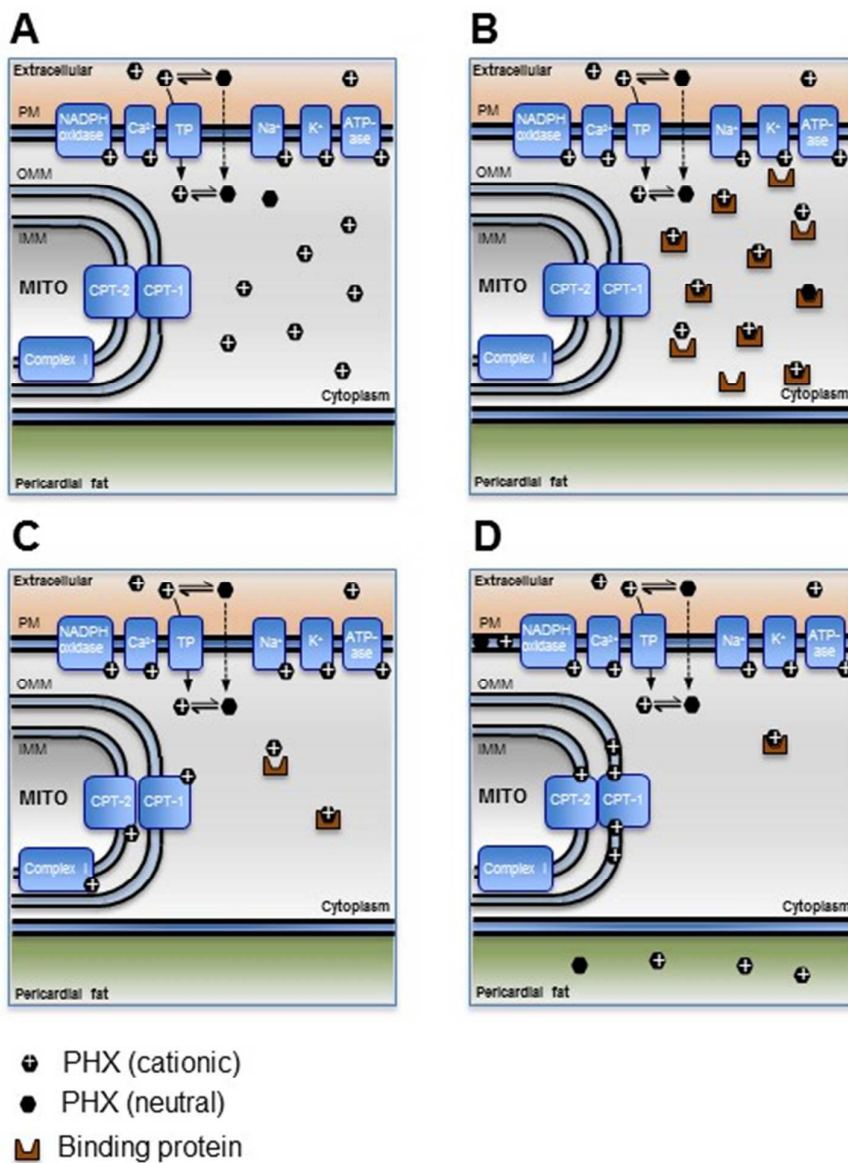
Drug	IUPAC name	Structure	logP	Primary target
Verapamil	(RS)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]-2-(propan-2-yl)pentanenitrile		3.8	L-type Ca <sup>2+</sup> channel
Nifedipine	3,5-dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate		2.2	L-type Ca <sup>2+</sup> channel
Disopyramide	(RS)-4-[bis(propan-2-yl)amino]-2-phenyl-2-(pyridin-2-yl)butanamide		2.6	Na <sup>+</sup> channels
Procainamide	4-amino-N-[2-(diethylamino)ethyl]benzamide		0.9	Na <sup>+</sup> channels
Flecainide	(RS)-N-(piperidin-2-ylmethyl)-2,5-bis(2,2,2-trifluoroethoxy)benzamide		3.8	Na <sup>+</sup> channels
Etomoxir	ethyl 2-[6-(4-chlorophenoxy)hexyl]oxirane-2-carboxylate		4.5	CPT
Perhexiline	2-(2,2-dicyclohexylethyl)piperidine		6.2	See Table 1
Oxfenicine	(2S)-2-amino-2-(4-hydroxyphenyl)acetic acid		0.2	CPT?
Dofetilide	N-[4-(2-[[2-(4-methanesulfonamidophenyl)ethyl](methyl)amino]ethoxy)phenyl]methanesulfonamide		2.1	K <sup>+</sup> channels
Amiodarone	{2-[4-(2-butyl-1-benzofuran-3-carbonyl)-2,-6-diiodophenoxy]ethyl} diethylamine		7.6	K <sup>+</sup> channels



**Figure 1. Endogenous inhibition of CPT-1 by malonyl-CoA**

Studies using  $^{13}\text{C}$ -radiolabelled etomoxir as a probe for CPT distribution revealed that in the heart, CPT-1 is expressed to higher levels than CPT2.<sup>50</sup> This raises the possibility that not all CPT1 is 'paired' with CPT2 as is depicted here. CPT-1B is the predominant cardiac isoform and is more sensitive to malonyl co-A inhibition than CPT-1A ( $\text{IC}_{50}$  of  $0.03\mu\text{M}$  vs.  $0.3\text{-}1\mu\text{M}$ , respectively)<sup>49</sup>.

GLUT, glucose transporter; G6P, glucose-6-phosphate; LDH, lactose dehydrogenase; FATP, fatty acid transporter protein; MCT, monocarboxylase transporter; PDC, pyruvate dehydrogenase complex,  $\beta\text{-ox}$ ,  $\beta$ -oxidation; CPT-1, carnitine palmitoyltransferase 1; CPT-2, carnitine palmitoyltransferase 2; CTP, citrate transporter protein; ACC, acetyl-CoA carboxylase; MCD, malonyl-CoA decarboxylase; IMM, inner mitochondrial membrane; OMM, outer mitochondrial membrane.



**Figure 2. Modes of cellular accumulation of PHX**

At pH7.4 approximately 99.9% of PHX would exist in the cationic form in equilibrium with approximately 0.1% as the neutral species. As described in section 5, organic ion transporter proteins (TP) probably play an important role in the active uptake and intracellular accumulation of PHX, as has been described for amiodarone.<sup>113</sup>

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3 (A) Increased cytoplasmic [PHX] and high intracellular concentrations of 'free' (unbound)  
4 PHX would not be possible under equilibrium conditions if plasma [PHX] was maintained at  
5 much lower levels.  
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9 (B) Cellular accumulation of PHX could occur if PHX bound with reasonably high affinity to a  
10 cytoplasmic target / binding partner.  
11

12  
13 (C) Selective accumulation of PHX by direct binding to its reported targets (see Table 1).  
14

15 (D) Heterogeneous distribution of PHX via partitioning of neutral and cationic species of drug  
16 into mitochondrial membranes which promotes high localized concentrations around CPT-1.  
17 Some [PHX] in the cytoplasm available to interact with surface membrane ion channels and,  
18 over time, accumulate in pericardial fat.  
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