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Published August 29, 2016

Thermodynamic analysis questions claims of improved cardiac efficiency by dietary fish oil

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Studies in the literature describe the ability of dietary supplementation by omega-3 fish oil to increase the pumping efficiency of the left ventricle. Here we attempt to reconcile such studies with our own null results. We undertake a quantitative analysis of the improvement that could be expected theoretically, subject to physiological constraints, by posing the following question: By how much could efficiency be expected to increase if inefficiencies could be eliminated? Our approach utilizes thermodynamic analyses to investigate the contributions, both singly and collectively, of the major components of cardiac energetics to total cardiac efficiency. We conclude that it is unlikely that fish oils could achieve the required diminution of inefficiencies without greatly compromising cardiac performance.

Introduction

Over the past few decades, two major rubrics concerning the health benefits of fish oils have arisen and captured both the scientific and popular imagination. The first of these suggests that a diet high in polyunsaturated fish oils diminishes the incidence of coronary artery disease. The initial evidence in support of this claim is attributed to the Danish investigators Bang and Dyerberg (Bang and Dyerberg, 1972; Bang et al., 1976), although their investigation contained no autopsy evidence and was based only on dietary surveys. Moreover, their surveys revealed that the "Eskimo diet" was typically high in saturated fats arising from the blubber of marine mammals. Recently, Fodor et al. (2014) comprehensively reviewed the evidence and concluded that a diet high in polyunsaturated fish oils has been promoted and sustained over the intervening decades, despite a dearth of supporting evidence.

The second thread is the more recent claim that a diet high in omega-3 fish oils can dramatically increase the pumping efficiency of the left ventricle, either by increasing its capacity for external work, with little or no change of oxygen consumption, or by a minimal effect on pressure-volume work in the face of decreased oxygen consumption. This thread commenced with a 2002 publication demonstrating that, at optimal filling pressure (10 mmHg) and an afterload of 75 mmHg, the total efficiency of the in vitro, blood-perfused, working heart increased from ∼4% in rats fed a standard diet to just over 10% in those fed a diet rich in omega-3, a 2.5-fold improvement (Pepe and McLennan, 2002). However, attention should be drawn to the very low baseline value of 4% (the mean from 10 control animals fed a standard chow diet). That publication was followed, five years later, by one showing that the total cardiac efficiency increased approximately linearly with dietary concentration of fish oil after a 6-wk high-fat diet. In that study, fish oil concentrations of 0%, 3%, 6%, and 12% were examined, while total fat content (saturated plus unsaturated) was held constant at 12% (see Fig. 1). Total efficiency increased progressively from ∼2% to ∼16%, an eightfold increase (Pepe and McLennan, 2007). Again we emphasize the very low value of total efficiency (2%) in the absence of dietary fish oils (or, equivalently, in the presence of 12% saturated fats). Interestingly, the impressive increase in efficiency with concentration of dietary fish oil mirrored an equivalent decline in VO_2. That is, pressure-volume work remained nearly constant, independent of diet. A subsequent study by the same group examined the benefits of dietary fish oil on the hearts of rats rendered hypertrophic by a 15-wk period of constriction of the abdominal aorta (McLennan et al., 2012). Once again, the cardiac benefits of dietary fish oil revealed themselves in measurements of isolated whole-heart energetics: 25% and 75% increases of total efficiency in the normotrophic (control) and hypertrophic groups, respectively. Note that these relatively modest increases

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The Rockefeller University Press $30.00
J. Gen. Physiol. 2016 Vol. 148 No. 3 183–193
www.jgp.org/cgi/doi/10.1085/jgp.201611620

Published August 29, 2016

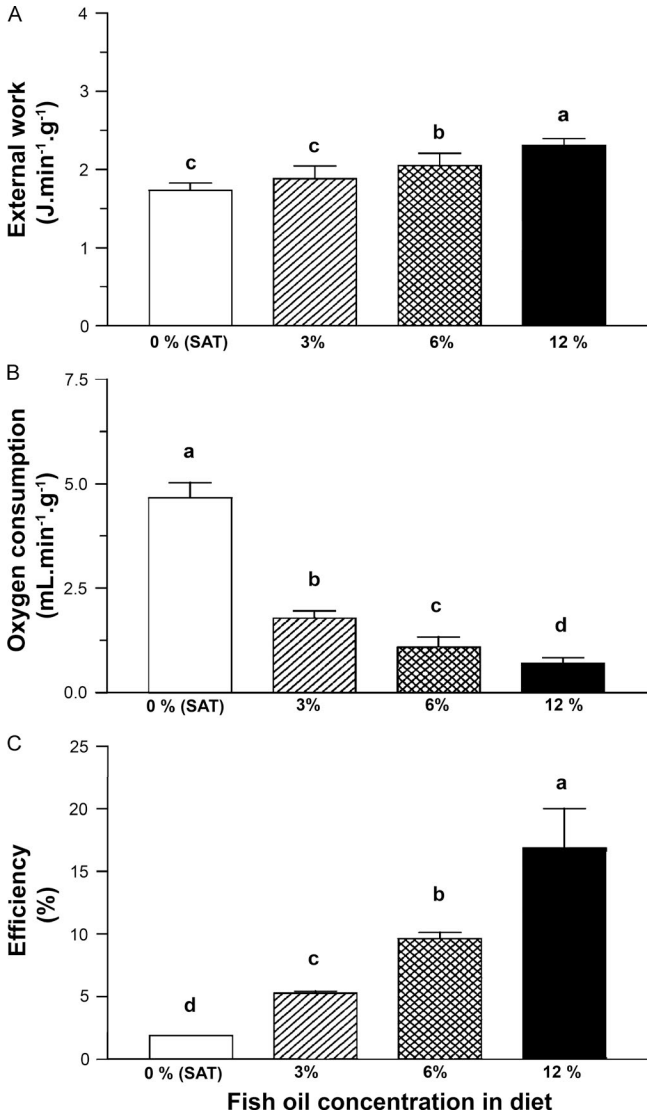

Figure 1. **External work, myocardial oxygen consumption, and cardiac energy utilization efficiency.** (A–C) External work (A), myocardial oxygen consumption (B), and cardiac energy utilization efficiency (C) during normoxic baseline function of the erythrocyte-perfused, isolated working hearts of rats that had been subjected to a 6-wk diet high in saturated animal fats (SAT) followed by a further 6 wk on diets of either saturated fats (0% SAT) or fish oil (FO) supplementation. Values are means ± SD, n = 10. Bars without a common letter differ, $P < 0.05$. Reproduced from Fig. 1 of Pepe and McLennan (2007) with permission of *The Journal of Nutrition* via RightsLink.

occurred above baseline values of 4% and 7.5%, respectively. Bolstering these results from animal studies is one showing the benefits of dietary fish oil supplementation in trained cyclists (Peoples et al., 2008): reductions of steady-state heart rate and whole-body VO_2 during submaximal exercise.

However, beneficial effects of fish oil diets have not been universally reported. Goo and colleagues (Goo et al., 2014a,b) fed rats on diets mimicking those used by Pepe and McLennan (2002) and found no effect on any parameter of cardiac function and, in particular, on the mechanical efficiency of either isolated RV trabeculae or isolated, saline-perfused rat hearts at 32°C (Goo et al., 2014a) or in saline-perfused hearts at 37°C (Goo et al., 2014b). Fig. 2 shows an example of these findings from the latter study under both variable preload (at a fixed afterload of 75 mmHg, Fig. 2 A) and variable afterload (at fixed preload, Fig. 2 B).

The results shown in Fig. 2, as well as comparable published results (Goo et al., 2014a,b), appear incompatible with those described above (Pepe and McLennan, 2002, 2007). This incompatibility has prompted us to pose the following question: What is the maximal theoretical limit of the increase in contractile efficiency of the heart that could be achieved as a consequence of any intervention? We address this question by exploiting the detail-independent virtue of thermodynamics. To do that, we first review the basic mechano-energetics of the heart.

Fundamentals of cardiac energetics

The total energy change of a chemical reaction taking place at constant pressure, ΔH, is known as the enthalpy change and is the appropriate concept for consideration of biochemical reactions occurring in cells. In whole-heart studies in which VO_2 is measured, ΔH is given by the energetic equivalent of oxygen (commonly considered to be ~20 kJ/liter or 448 kJ/mol); in thermal studies

$$\Delta H = W + Q, \quad (1)$$

where ΔH signifies the enthalpy gained by the heart from its surroundings, W is the work done on the heart by its surroundings, and Q is the heat gained by the heart from its surroundings. As is intuitively apparent, these terms are invariably negative relative to the heart, in that the heart performs pressure-volume work (W) on its surroundings and loses heat (Q) to its surroundings, these two negative quantities being equal to the enthalpy lost by the heart to its surroundings. Eq. 1 expresses the first law of thermodynamics.

The performance of work, by membrane ion pumps and the actin–myosin cross-bridges of the myofilaments, is funded directly by the hydrolysis of ATP. Under intracellular conditions at body temperature, the change of Gibbs free energy involved in ATP hydrolysis (ΔG_{ATP}) is negative (i.e., free energy is lost as ATP is hydrolyzed) and is approximately equal to 60 kJ/mol in magnitude. This free energy loss is somewhat larger than the corresponding enthalpy loss (ΔH_{ATP}) because this reaction gains entropy, inextricably but reversibly, through the hydrolysis of ATP into its end products: ADP and Pi (inorganic phosphate). This qualitative description can be quantified and formalized as the second law of thermodynamics:

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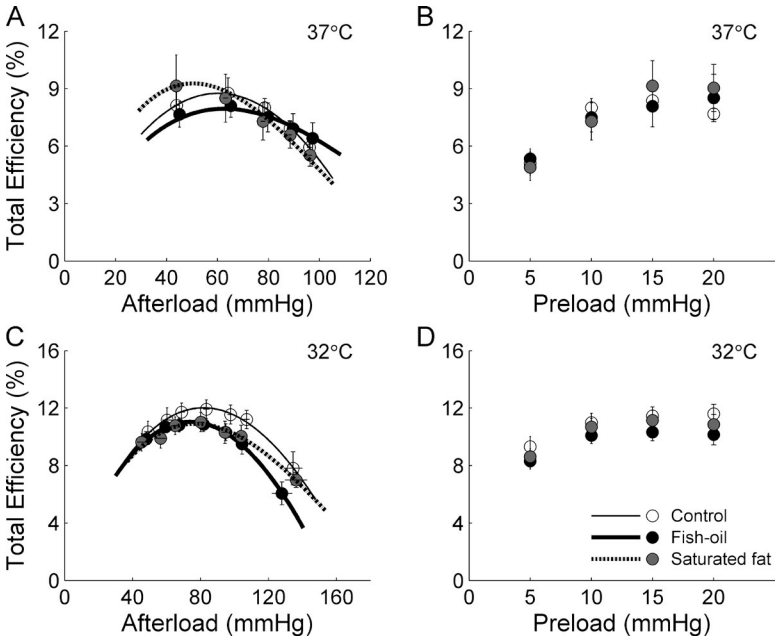

Figure 2. **Total efficiency of isolated, saline-perfused hearts at 37°C and 32°C from rats fed isocaloric diets.** W = pressure-volume work; ΔH = molar enthalpy of oxygen. (A and C) Effect of variable afterload at a fixed preload of 10 mmHg. (B and D) Effect of variable preload at a fixed afterload of 75 mmHg. No differences among diets in any panel. Mean ± SE is shown. Reproduced from Fig. 2 of Goo et al. (2014a) and Figs. 4 and 5 of Goo et al. (2014b), under the Rights of Authors of Physiological Society Articles and Physiology Reports, respectively.

$$\Delta H_{ATP} = \Delta G_{ATP} + T\Delta S_{ATP}, \quad (2)$$

where T is absolute temperature (K) and ΔS_{ATP} is the reversible gain of entropy (kJ mol^{-1} K^{-1}) during the hydrolysis event.

Not all of the free energy that is available from ATP hydrolysis (ΔH_{ATP}) can be captured by the molecular machinery of the cell; that is, the conversion of the free energy of ATP hydrolysis into work is not 100% efficient. (Indeed, it could not be if the associated reactions are to proceed at nonzero velocity.) Using the Greek letter η to denote thermodynamic efficiency, we can now restate the second law expression of Eq. 2:

$$\begin{aligned} \Delta H_{ATP} &= W + (1-\eta)\Delta G_{ATP} + T\Delta S_{ATP} = \\ W &+ Q_{irrev} + Q_{rev} = W + Q, \end{aligned} \quad (3)$$

in accord with the first law. It remains merely to observe that the two distinct heat terms in Eq. 3 are opposite in sign, i.e., Q_{irrev} is the heat produced (lost) by the heart through free energy dissipation (involving entropy creation), whereas Q_{rev} is the heat gained by the heart through the process of entropy exchange; the irreversible component of heat (Q_{irrev}), when divided by the temperature, quantifies the extent of entropy creation, whereas Q_{rev}/T comprises entropy exchange. In the particular case of ATP, the same amount of heat (Q_{rev}), at the same temperature, is generated at the mitochondrial ATP synthase during the act of reassembling ATP from its hydrolysis products, thereby "closing the loop" of entropy exchange as the mitochondrion loses entropy through ATP synthesis. The net heat generated by mitochondria is known in the muscle field as "recovery heat." It is assumed to be present in all the conceptually distinct components of aerobic heat production discussed below (see Eq. 5).

It is evident from Eq. 3 that thermodynamic efficiency quantifies the proportion of the Gibbs free energy, inherent in ATP, that is used to perform work at the molecular level. Regrettably, it is not possible to measure η directly. Hence, experimentalists have devised a more intuitive measure to describe efficiency in the macroscopic domain:

$$\varepsilon = W/\Delta H = W/(W + Q). \quad (4)$$

For our purposes, W connotes macroscopic work, either the pressure-volume moiety by the intact heart or the force-length equivalent by its isolated tissues. In anticipation of the discussion that follows, we note that, for any value of W, as Q approaches 0 efficiency approaches 1.0.

At the global level, cardiac heat production arises from three conceptually distinct components: basal metabolism (Q_B), activation metabolism (Q_A), and cross-bridge cycling ($Q_{X\text{-}b}$). We define total cardiac efficiency as

$$\varepsilon_T = W/(W + Q_B + Q_A + Q_{X\text{-}b}). \quad (5)$$

Under suitable experimental conditions, designed such that basal metabolic rate can be discounted, then we speak of mechanical efficiency:

$$\varepsilon_{Mech} = W/(W + Q_A + Q_{X\text{-}b}). \quad (6)$$

Partitioning of cardiac enthalpy production. The heart is a molecular machine. Its myocytes directly convert the Gibbs free energy of ATP hydrolysis (ΔG_{ATP}) into force development and shortening of the sarcomeres as well as the pumping of cations during the resto-

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ration of sarcolemmal gradients of Na^+ and K^+ and the sequestration of Ca^{2+} back into the SR after the activation of contraction. The metabolic cost of the collective restoration of ionic gradients is known as "activation heat" (Q_A; Hill, 1949). Its value is typically found to comprise some 20–25% of total cardiac metabolism. The brief flood of Ca^{2+} from the SR triggers the myosin-activated, ATP-dependent cycling of the actomyosin cross-bridges and subsequent performance of external (pressure-volume or force-length) work, accompanied by the evolution of heat ($Q_{X\text{-}b}$). This heat arises from two distinct entropic sources: (1) the entropy created as a result of the thermodynamic inefficiency of the mechanical transduction, whereby not all of ΔG_{ATP} is captured for work by the cross-bridges (Eq. 3), and (2) the entropy exchanged with the surroundings through hydrolysis of ATP and that will subsequently be restored during regeneration of ATP from ADP and Pi by the mitochondrial ATP synthase. Each of the ATP-consuming ionic and mechanical events takes place in the cytoplasm of the myocyte. Collectively, their consumption of ATP comprises the initial enthalpy:

$$\Delta H_I = W + Q_{X\text{-}b} + Q_A. \quad (7)$$

There is sufficient creatine phosphate in the myocytes to provide ATP via the Lohmann reaction to power the ionic pumps and cross-bridges for only a few contractions. Thereafter, if performance is to continue, ATP must be supplied by the mitochondria, via oxidative phosphorylation of metabolic substrates. The heat generated by restoration of creatine phosphate and ATP consumed during the initial (ionic and cross-bridge) processes is labeled recovery enthalpy (ΔH_R). In isolated amphibian skeletal muscle contracting at low temperature, initial enthalpy is temporally distinct from the subsequent recovery enthalpy (Hartree and Hill, 1922). In cardiac muscle contracting at body temperature, these two thermal events present themselves essentially contiguously, and their separation requires the use of mathematical deconvolution techniques. Nevertheless, they remain conceptually distinct and experimentally separable. The current consensus is that $\Delta H_R/\Delta H_I = 1.2$ (Barclay and Widén, 2010), thereby revealing that somewhat more than one half of cardiac enthalpy production is restorative in nature.

It remains only to consider the basal component of cardiac metabolism. When studying isolated tissue preparations, the basal state is readily achieved by terminating electrical stimulation. When the isolated whole-heart is under investigation, some sort of ionic or pharmacological intervention is required to prevent both activation and cross-bridge cycling. In either case, basal enthalpy (ΔH_B) is found to be of comparable magnitude with that of activation enthalpy, comprising some 20–25% of total enthalpy (Gibbs and Loiselle, 2001).

We are now in a position to summarize the components of total cardiac enthalpy production, which we do graphically (Fig. 3) and conceptually (Eq. 8):

$$\Delta H_T = \Delta H_I + \Delta H_R + \Delta H_B. \quad (8)$$

In this expression, ΔH_I is defined in Eq. 7, whereas ΔH_B has been explicitly omitted from Eq. 6, which provides the definition of mechanical efficiency (ε_{Mech}).

Wherein and to what extent could efficiency be increased?

We consider this question in four ways. First, using the assumed proportions of each of the three components of total cardiac enthalpy production (Fig. 3 and Eq. 8), we sequentially set each to 0, assuming a starting value of total cardiac efficiency of 0.20. This is an admittedly harsh approach because the assumed starting value of efficiency (0.20) is at or near the top of the range observed experimentally. Furthermore, the complete elimination of a single source of heat is physiologically unrealizable. Hence, second, we relax the constraints by allowing any nonzero value for the initial efficiency (i.e., of a control group) and quantify the extent to which the output of heat would need to be reduced to achieve any arbitrary final value of efficiency (i.e., of an intervention group) up to 0.20. Once again, we do this sequentially and separately for the three components. Third, we relax the constraints further by combining all three components of heat production. Finally, we examine the consequence, to the calculation of cardiac efficiency, of measurement errors in the experimental determinations of work and heat.

(a) Consideration of each energetic component separately. The partitioning of total enthalpy into its distinct, experimentally separable, components allows estimation of the contribution that each, in turn, might make to increasing total efficiency.

(i) Recovery metabolism. We commence by noting that all four components of total enthalpy production (ΔH_T) in Fig. 3 include both initial (ΔH_I) and recovery (ΔH_R) metabolism and recall that the $\Delta H_R/\Delta H_I$ ratio is 1.2 so that ΔH_R accounts for ~55% of ΔH_T. The sources of inefficiency in the sequence of steps comprising recovery metabolism, i.e., mitochondrial oxidative phosphorylation, are not yet known in detail but may include proton leakage across the inner mitochondrial membrane (Goo et al., 2013; Pham et al., 2014; Power et al., 2014), proton slippage at the ATP synthase (Brown, 1992), the production of reactive oxygen species (Chouchani et al., 2014), and electron leakage, espe-

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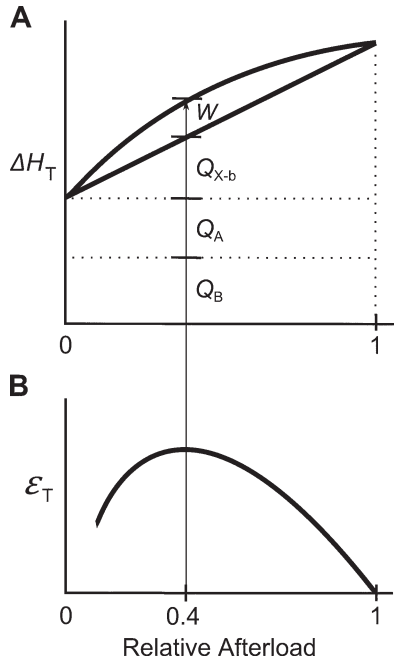

Figure 3. **Schematic diagram of the components of total enthalpy expenditure.** (A and B) Total enthalpy expenditure, ΔH_T: W = work, $Q_{X\text{-}b}$ = cross-bridge heat, Q_A = activation heat, Q_B = basal heat (A); and total efficiency, ε_T (B), as functions of afterload. The vertical line at 0.4 relative afterload coincides with the peak value of total efficiency, consistent with the experimental data shown in Fig. 2 and corresponding to 60–80 mmHg.

cially at complexes I and III (Jastroch et al., 2010; Divakaruni and Brand, 2011).

The first attempt to measure the efficiency of recovery metabolism in striated muscle was made by Lou et al. (2000) using white muscle fibers from the dogfish. This preparation was chosen because metabolic recovery from contraction has only a minor contribution from anaerobic processes. In that regard, it is similar to cardiac muscle. Those authors found the efficiency of recovery metabolism to average 84% (n = 29 fibers). The only study using cardiac muscle is that of Barclay and Widén (2010). These authors reported a value for the efficiency of recovery metabolism of 72% (n = 9) in left ventricular, murine, papillary muscles. In the interest of caution, we will accept the latter value to calculate the consequence to total cardiac efficiency (ε_T) if a dietary regimen of fish oils could effect 100% efficiency. To provide realistic numeric estimates, we assume that the mitochondrial efficiency of control hearts is 0.72 and the total efficiency of the heart in vivo is 0.20 (Gibbs et al., 1967; Neely et al., 1967; Gibbs, 1978; Gibbs and Barclay, 1995).

If mitochondrial inefficiency → 0, then total efficiency → 0.20 × 2.2/(1 + 0.72*1.2) = 0.24.

Thus, complete elimination of all sources of mitochondrial inefficiency would increase total efficiency from 20% to 24%. This is not only a paltry increase vis-à-vis the several-fold increments reported in the literature (see Fig. 1, for example), but would be achieved at the cost of halting ATP production (Chapman and Loiselle, 2016).

(ii) Basal heat (Q_B). The rate of basal heat production by cardiac muscle is exceptionally high vis-à-vis that of skeletal muscle (for a review, see Gibbs and Loiselle [2001]), comprising upwards of one quarter of the total enthalpy expenditure of the heart. Given that (a) the rate of myocardial protein turnover is high, (b) both degradation and synthesis require energy expenditure, and (c) both the rate of basal heat production and (c) the rate of protein turnover vary inversely with species body size, protein flux is a likely candidate, but in the absence of firm experimental evidence, this suggestion must remain speculative. Hence, it would be premature to suggest a putative reduction of ΔH_T from this source. More likely is a role for proton flux (Loiselle, 1987; Divakaruni and Brand, 2011), reflecting the uncoupling of oxidative phosphorylation, possibly as a source of heat for the maintenance of homeothermy. Evidence that fatty acids enhance mitochondrial proton leak (Jastroch et al., 2010), as well as reactive oxygen species production (Yu et al., 2014), may provide an explanation for the observation that acute perfusion of ex vivo murine hearts with the fatty acid palmitate invokes a near doubling of the rate of basal oxygen consumption (Boardman et al., 2011), whereas palmitate supplementation of a high-fat diet has been shown to reduce the efficiency of ex vivo rat hearts by some 30% (Cole et al., 2011). If fish oils have the same effect, perhaps via reduction of the apparent K_m for ADP (Herbst et al., 2014), then a diminution of total cardiac efficiency would occur. Nevertheless, we quantify the maximal increment of efficiency if fish oils were to eliminate basal metabolism completely.

If $-\Delta H_B \rightarrow 0$, then total efficiency: $\varepsilon_T \rightarrow 0.20/(1 - 0.25) = 0.27$.

That is, elimination of basal metabolism, estimated to account for 20% of total metabolism at a relative afterload of 1.0, but 25% at the optimal relative afterload of 0.4, would increase total metabolic efficiency from 20% to 27%, independent of the assumed value of W (in this case: 0.20).

(iii) Activation heat (Q_A). Whereas the metabolic cost of activation in both healthy and diabetic mouse hearts has been shown to be increased by both chronic and acute provision of palmitate (How et al., 2005, 2006), we nevertheless assume that Q_A is fixed at 0.2 ΔH_T, as shown in Fig. 3. Under that assumption, the maximal conceivable increase of total cardiac efficiency, by eliminating activation heat, would be as follows.

If $Q_A \rightarrow 0$, then total efficiency: $\varepsilon_T \rightarrow 0.20/(1 - 0.25) = 0.27$.

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As is the case of basal heat production (previous section), the scope for improvement of total cardiac efficiency by elimination of activation heat (without elimination of activation!) would be meagre.

(iv) Cross-bridge heat ($Q_{X\text{-}b}$). We assume that cross-bridge heat arises primarily from inefficient conversion of the Gibbs free energy of ATP hydrolysis during cross-bridge cycling. Such inefficiency is most readily visualized by reference to the formalism of Eisenberg and colleagues (Eisenberg and Hill, 1978; Eisenberg et al., 1980); we adopt the approach of those authors. An individual cross-bridge has a limited "reach" over which it can make and maintain attachment. If it detaches prematurely, then it will not have performed its maximal work potential, and it will have squandered some fraction of the Gibbs free energy of its attendant ATP molecule. At the other extreme, if its detachment is tardy and it is drawn past its equilibrium position ($x = 0$ in Huxley's formalism [Huxley, 1957]), then it will resist shortening as its direction of force changes from pull to push (Barclay, 1999), an entirely counterproductive situation. In either case, some proportion of ΔG_{ATP} is wasted. The consequences of such microscopic events can be scaled up to the macroscopic domain.

By attributing 20% of total cardiac enthalpy production to each of the basal, activation, and work components, independent of afterload (see Fig. 3), then, at a relative afterload of 0.4, 40% is necessarily assigned to cross-bridge heat. If all cross-bridge heat could be eliminated as a consequence of consuming omega-3 fish oils, then a 1.6-fold increase of cardiac efficiency would be obtained.

If $Q_{X\text{-}b} \rightarrow 0$, then total efficiency: $\varepsilon_T \rightarrow 0.20/(1 - 0.4) = 0.33$.

However, the second law of thermodynamics, as expressed by the probability isotherm (Chapman et al., 2011; Chapman and Loiselle, 2016), dictates that such a putative extent of improvement of total efficiency could be achieved only at equilibrium, i.e., in the absence of net cycling of cross-bridges, ergo, in the absence of either microscopic or macroscopic work.

(v) Work (W). Macroscopic cardiac work (whether pressure-volume or force-length) is achieved by the shortening of sarcomeres, in turn achieved by rotation of attached (i.e., force-producing) cross-bridges. What is the scope for increasing unitary cross-bridge work performance?

Barclay and colleagues (Barclay et al., 2010; Barclay, 2015), using the theoretical framework developed by Huxley and Simmons (Huxley and Simmons, 1971, 1973), have inferred cross-bridge properties from the energetics of skeletal muscle. By considering the ultra-slow, ultra-efficient rectus femoris muscle of the tortoise, they estimate that the maximum work that a cross-bridge can perform in a single attachment to actin is some 40 zJ (where z [the abbreviation for zepto] is the SI unit denoting 10^{-21}). Under the assumption that one ATP molecule is hydrolyzed per cross-bridge event and by approximating the Gibbs free energy of a single ATP molecule to be 100 zJ (the quotient of 60 kJ/mol ATP and Avogadro's number), they infer a cross-bridge efficiency of 0.4. In contrast, cross-bridges of cardiac muscle perform around 20 zJ per cycle. Thus, even if cardiac cross-bridges could extract as much work from the free energy of ATP hydrolysis as tortoise skeletal muscle (but at the cost of greatly slowed performance), the most that could be expected would be a doubling of total cardiac efficiency. Needless to say, no tortoise-like reduction of kinetics (rates of rise or fall of isometric twitch force) has been reported as a consequence of consumption of fish oils.

(b) Relaxation of the constraint that $\varepsilon_1 = 0.2$. In the preceding analysis, we have made use of Eq. 5, sequentially setting each of the heat terms in the denominator to 0, to calculate the effect on total efficiency. In doing so, we adopted a starting or control value of $\varepsilon_1 = 0.20$ and an optimal relative afterload of 0.4 (Fig. 3). In Fig. 4 (A and B), we extend this analysis by allowing ε_1 to take on any value <0.2 and plotting the resulting improved efficiency: ε_2. As can be seen in either panel, the lower the starting efficiency (ε_1), the greater the resulting efficiency (ε_2) when either cross-bridge heat (blue line) or either of basal or activation heat (black line) are eliminated. But, as is also evident, even an improbably low starting value ($\varepsilon_1 = 0.02$) is incapable of achieving a final doubling of total cardiac efficiency (red line), let alone a quadrupling or more, as has been claimed for dietary fish oils (Fig. 1).

(c) Consideration of energetic components collectively and simultaneously. In both of the preceding analyses (Fig. 4, A and B), we have focused on the influences of individual thermal components. We now present a more realistic approach by allowing all components to vary simultaneously, while accepting any value for the efficiency of a nonintervention (control) group. We commence by noting that, in Fig. 1, despite the range of total cardiac efficiency from 2% to 16%, to a first approximation, work output was constant. We exploit this approximation of constancy of work output to derive an expression for the amount by which the total output of heat would have to increase (at constant work load) to achieve selected values of efficiency.

Under the approximation that $W_1 = W_2$, it follows that

$$\frac{Q_2}{Q_1} = \frac{\varepsilon_1 * (1 - \varepsilon_2)}{\varepsilon_2 * (1 - \varepsilon_1)}. \quad (9)$$

Graphs of this relationship, for five different values of ε_1 (0.02, 0.04, 0.08, 0.15, and 0.20) are presented in

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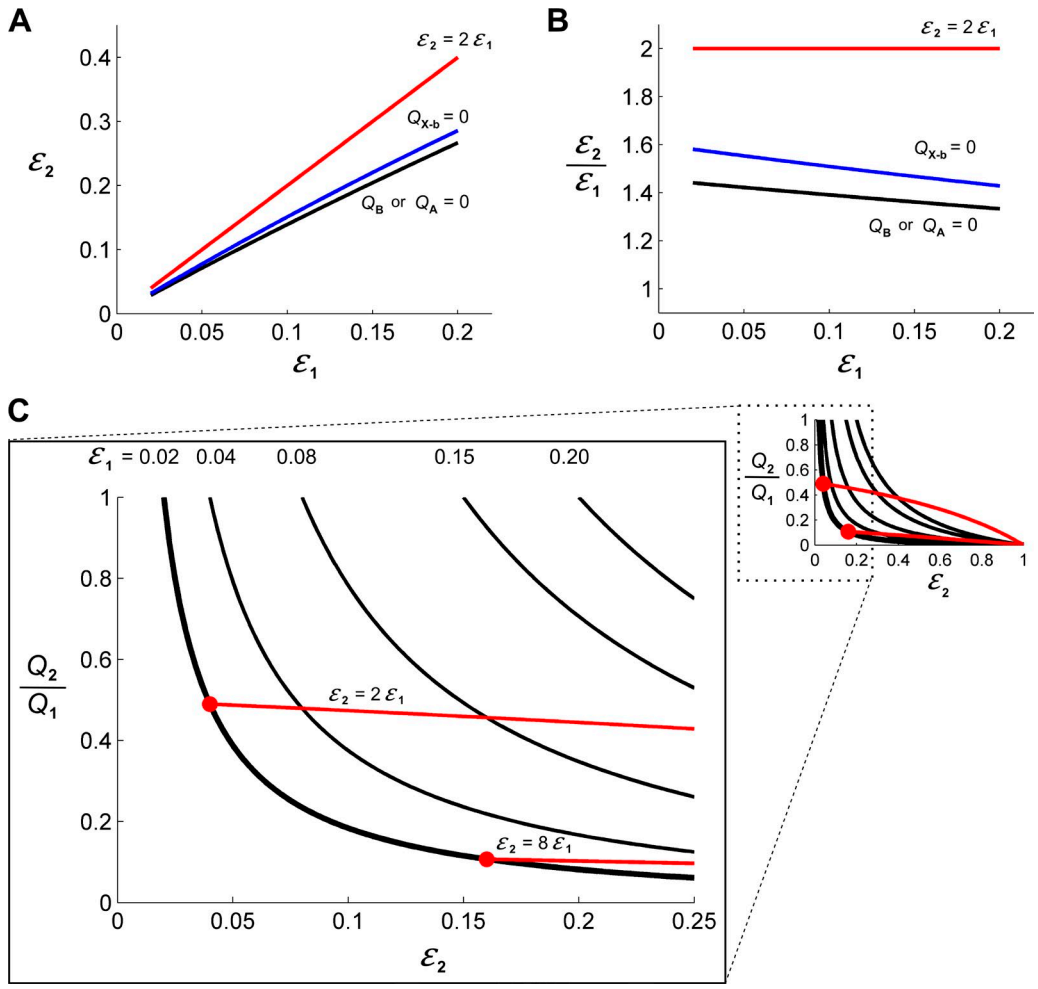

Figure 4. **Expected consequences to efficiency from setting selected thermal components to zero and, conversely, to diminution of heat production in order to achieve specified increments of efficiency.** (A and B) Effect on absolute (A) and relative (B) efficiencies resulting from setting, independently, each individual thermal component to zero. The red lines indicate requirements to achieve doubling of efficiency (mimicking the minimal increment of efficiency shown in Fig. 1 C). (C) Required diminution of total heat production required to improve total cardiac efficiency (at fixed work load) from selected values of ε_1 (0.02, 0.04, 0.08, 0.15, and 0.20) to any required value of ε_2 indicated on the abscissa. The red lines show that, commencing from $\varepsilon_1 = 0.02$ (corresponding to the control group in Fig. 1), heat output would need to reduce by 50% to double efficiency and by 90% to increase it eightfold (to mimic the results shown in Fig. 1 C). The boxed region of the inset encloses the range of estimates of total cardiac efficiency typically reported in the literature.

Fig. 4 C. They allow quantification of the extent to which heat output must diminish to increase efficiency from its value in the control group (ε_1) to its value in an intervention group (ε_2). For example, if the mean efficiency of control hearts is initially 0.02, then to increase it to 0.04 (at constant work load), heat output would need to diminish by 50% (red line labeled $\varepsilon_2 = 2\varepsilon_1$). To increase it to 0.16 would require heat output to decrease by 90% (red line labeled $\varepsilon_2 = 8\varepsilon_1$). For any required increment of efficiency (from ε_1 to ε_2), the extent of diminution of heat increases with ε_1, as shown in the inset, where the boxed region encloses the range of typical estimates of total cardiac efficiency as reported in the literature.

(d) A holistic approach. An alternative global approach, which avoids the assumption of constancy of work, is to consider the effect of simultaneous changes to the estimates of both work and heat output. This is done by deriving an expression for their second-order partial derivatives with respect to efficiency. Given that

$$\varepsilon = \frac{W}{W + Q},$$

it follows that

$$\frac{\partial^2 \varepsilon}{\partial W \partial Q} = \frac{(W - Q)}{(W + Q)^3}.$$

The resulting 3-D plot is presented in Fig. 5, where the error in estimation of ε (vertical axis) is shown for any combination of W and Q resident on the surface. Note that the relative scaling on the Q axis is from 0.05 to 0.40, whereas that on the W axis is from 0.02 to 0.10.

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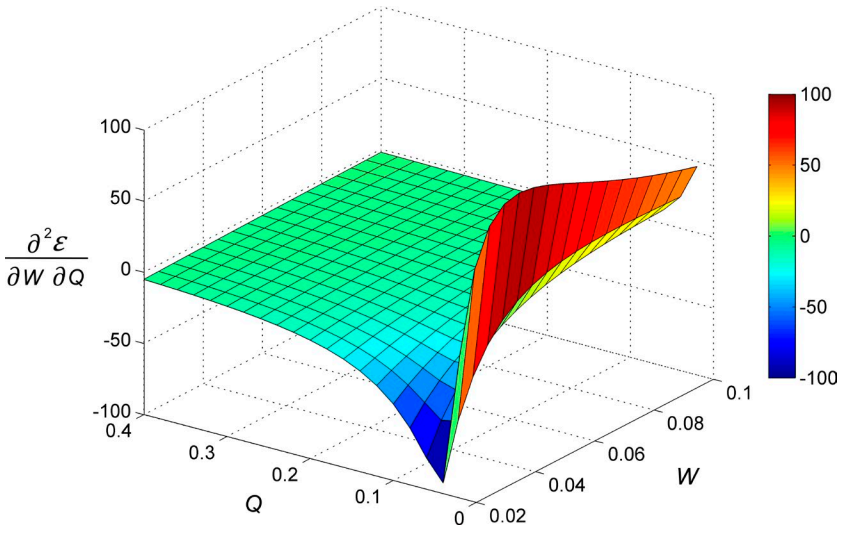

Figure 5. **3-D surface plot showing the consequences, to calculated values of total cardiac efficiency (ε), of errors of measurement in estimating work (*W*) and heat (*Q*).**

These values were chosen to span regions of ε from near 0 to 0.20, which occurs at the (*W*, *Q*) coordinates: (0.1, 0.4). That is, 0.1/(0.1 + 0.4) = 0.20, the location at the most distant corner of the surface as viewed in its present orientation. Note that, over much of the surface, the *z* coordinate is near zero, indicating that minimal effects on the calculated value of ε can be expected in the face of modest measurements errors in either *W* or *Q*. However, as *Q* approaches zero (as is necessarily the situation in Fig. 1, in response to progressive increases in the concentration of dietary fish oil), then the error in estimation of ε inflates in proportion to W^{-2}.

Discussion

Our collective investigations demonstrate that thermodynamic considerations place severe restrictions on the extent to which any intervention can be expected to increase the efficiency of contraction of the myocardium. Constraints are of two distinct sorts. In the first case, both basal metabolism (which, for reasons unknown, is inherently high in cardiac muscle) and activation metabolism (mandatory if any muscular event is to occur) are pure "overhead" costs, obligatory for the maintenance of life and the quickening of its pulse, respectively, but overheads, nevertheless. In the above development, we show that even complete elimination of these individual overhead costs would contribute only a modest increase of efficiency, regardless of whether the efficiency of the control group is initially high or low. More generally, we show that any mechanism proposed to increase efficiency, by decreasing inefficiency, is constrained by the need to expend Gibbs free energy if macroscopic movement is to occur. Consequently, our findings that dietary fish oils have no influence on cardiac efficiency (Fig. 2) cannot be reconciled with reports in the literature (Fig. 1, for example) to the contrary. In fact, from our consideration of thermodynamic constraints, we consider that no intervention is likely to achieve even a doubling of overall cardiac efficiency, a contention supported by the data shown in the main panel of Fig. 4 C. Whereas, in principle, it would seem possible to double efficiency, even from the improbably low control value of 2%, in practice this would require a halving of heat production. It is unclear where such a saving could be achieved, even if shared among mitochondrial recovery metabolism and the four components of initial metabolism, without severely comprising work output. However, in the interest of completeness, the inset of Fig. 4 C demonstrates that, by sufficient reduction of inefficiencies (i.e., heat production [Eq. 9]), perfect efficiency could be achieved by some intervention, independent of its value under control conditions. Nevertheless, the published reports (Pepe and McLennan, 2002, 2007) of striking improvements of total cardiac efficiency as a consequence of dietary enhancement with fish oils appear incompatible with the laws of thermodynamics, and we offer several putative attempts at reconciliation below.

Attempts at reconciliation. Despite our attempts to reconcile the disparate results presented in Figs. 1 and 2, by recourse to fundamental thermodynamic considerations, we have been unsuccessful. Nevertheless, we offer three additional possibilities.

(1) "Mismatch" of efficiency-afterload relations. In several places in the preceding discourse, we have drawn attention to the very low values of efficiency (2–4%) reported for hearts of animals placed on control diets and in Fig. 4 show the improbability of achieving the reported improvements even if commencing from such values. But we have further concerns, as follows. Over a century ago, Evans and Matsuoka (1915) used the blood-perfused heart-lung preparation to measure the total efficiency of the isolated dog heart, finding it

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to range from 8% to 18% as cardiac output was progressively increased. Half a century later, Neely et al. (1967) developed techniques to vary independently both the preload and afterload placed on the isolated working rat heart, while simultaneously measuring its rate of oxygen consumption, thereby allowing estimation of total cardiac efficiency. As preload was increased from 0 to 20 cm H_2O, efficiency increased from 4% to 17%. Note that 4% efficiency resulted from a preload of 0. In the same year of publication as Neely's seminal paper, Gibbs et al. (1967) achieved the first application of the thermometric method to the study of cardiac muscle, measuring the rate of heat production of isolated rat right-ventricular papillary muscles performing afterloaded contractions at room temperature. These authors reported a mean peak value of total efficiency of 19%, in remarkable agreement with that arising from the aforementioned whole-heart study. Gibbs et al. (1967) could likewise observe very low values of efficiency (1.6–5%; see their Table 2) but only by increasing the afterload to the point where the muscle could scarcely shorten and work output was negligible. Such behavior is implicit in Eq. 4, in the experimental data presented in Fig. 2, and in the hypothetical situation presented in Fig. 6. In the latter case, the peak efficiencies of hearts from both groups are identical, but the afterload is inappropriately high for the control group. It could, of course, be inappropriately low and achieve a comparable afterload-efficiency mismatch, resulting in underestimation of peak efficiency of the control group.

(2) Buffer perfusion versus blood perfusion. Buffer perfusion versus blood perfusion is the sole substantive difference of experimental protocols between our studies (as exemplified in Fig. 2) and those of Pepe and McLennan (Pepe and McLennan, 2002, 2007; as exemplified in Fig. 1). This difference can be ruled out as a likely contributor for four reasons. First, it is striking that, whatever advantage may have been conferred on the isolated rat heart, by the superior delivery of oxygen by blood, it clearly did not extend to its efficiency of performing pressure-volume work (Fig. 1 A). Second, any putative benefits of blood perfusion were highly selective, benefiting efficiency in the presence of fish oils but not in their absence. Third, under even the harshest of perfusion protocols (periods of ischemia followed by reperfusion), no difference was observed in either post-ischemia, steady-state, mechanical performance (Sandhu et al., 1993; Galiñanes et al., 1996) or tissue content of high-energy purines (Galiñanes et al., 1996). Fourthly, Duvelleroy et al. (1976) showed some 40 years ago in the isolated rat heart that, despite variations of work and oxygen consumption, cardiac efficiency remained resolutely independent of hematocrit values ranging from 0% to 40%.

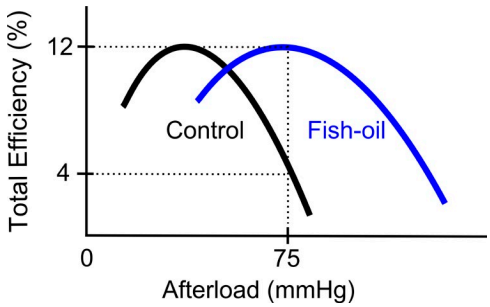

Figure 6. **Schematic comparison of differently located efficiency-afterload relations.** Note the identical afterloads and the identical true efficiencies (greatly underestimated for the control group).

(3) A "statistical" artifact. Suppose that animals of inherently low cardiac efficiency were consistently assigned to the control group. Using the value of $n = 10$ rats per group from Fig. 1, we assign a value of 0.25 for the probability of placing any particular animal in any particular group. Hence, the probability of assigning all 10 animals of low cardiac efficiency to a particular group is given by $(0.25)^{10} = 10^{-6}$, and the probability of assigning them to the control group would be further reduced to 0.25×10^{-6}. This is such an improbably small value that we rule out the possibility of its occurrence.

Postscript. As indicated in the Introduction, our study was motivated, in part, by the publication of Fodor et al. (2014), which largely debunks the presumed scientific basis on which a diet rich in omega-3 fish oils is believed to improve the health of the heart. We have since become equally disturbed by the publication of Ramsden et al. (2016), which casts grave doubts on the scientific basis on which the "diet-heart hypothesis" (which purports that a diet rich in vegetable-sourced linoleic acid reduces coronary heart disease and lowers serum cholesterol) has gained traction in the medical and popular media.

Summary

Our manuscript has been prompted by two recent developments. The first is the publication by Fodor et al. (2014), who revealed a largely incorrect and scientifically discomfiting history of the alleged health benefits of dietary fish oil supplementation. The second is our inability to reconcile the results of our own experiments with several published in the literature. We have pursued various possible theoretical explanations based on thermodynamic considerations, which, either singly or collectively, render reconciliation unlikely. But note that our purview is restricted to the contractile efficiency of the cardiac pump; regarding the numerous other claims of health benefits of dietary fish oils, we are agnostic.

Published August 29, 2016

ACKNOWLEDGMENTS

We acknowledge the generous funding of National Heart Foundation of New Zealand Project Grant #1601. Dr. J.-C. Han is supported by National Heart Foundation of New Zealand Research Fellowship #1611 and a Royal Society of New Zealand Marsden Fast-Start Grant (UOA1504).

The authors declare no competing financial interests.

Eduardo Ríos served as editor.

Submitted: 5 May 2016
Accepted: 9 August 2016

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