Thermodynamic Analyses Question Claims of Improved Cardiac Efficiency by Dietary Supplementation with Fish-Oil

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Condensed Title: Fish-oils and Cardiac Thermodynamics

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ABSTRACT

We have attempted to reconcile published reports of greatly improved pumping efficiency of hearts from rats receiving dietary supplementation with Omega-3 fish-oils with our own published null results. To that end, we have undertaken a quantitative analysis of the improvement that could be expected theoretically, subject to physiological constraints. We pose the question: “By how much could efficiency be expected to increase if inefficiencies could be eliminated?” Our approach utilises thermodynamic analyses, investigating 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.

KEY WORDS:

Omega-3 fish oils

Whole-heart efficiency

Cardiac muscle efficiency

Partitioning of cardiac energy expenditure

Cardiac muscle thermodynamics

INTRODUCTION

Over the past several 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 alleges that a diet high in polyunsaturated fish-oils diminishes the incidence of coronary artery disease. The initial evidence in support of this claim is invariably attributed to the Danish investigators Bang and Dyerberg (1972; 1976), despite the fact that their investigation contained no autopsy evidence and was based exclusively on dietary surveys. In fact, their surveys revealed that the 'Eskimo diet' was typically high in saturated fats arising from the blubber of marine mammals! It is to the great credit of Fodor *et al.*(2014), that their comprehensive review details how the notion that a diet high in polyunsaturated fish oils arose and 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 with 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 approximately 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). We draw attention to the very low 'baseline' value: 4% (the average from 10 control animals fed a standard chow diet). That publication was followed, five years later, by one showing that, following a six-week high-fat diet, total efficiency increased approximately linearly with dietary concentration of fish oil. 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 Figure 1). Total efficiency increased progressively from approximately 2% to approximately 16% - i.e., an 8-fold increase (Pepe and McLennan, 2007). Again we emphasise 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 of 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-week 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 occurred on 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 *et al.* 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). Figure 2 shows an example of these null findings from the latter study under both variable pre-load (at a fixed afterload of 75 mmHg, panel A) and variable afterload (at fixed pre-load, panel B).

There is obvious disparity between the results shown in Figure 2, as well as comparable published results (Goo *et al*., 2014b, a), and those described above (Pepe and McLennan, 2002, 2007). Such *prima facie* irreconcilability has prompted us to pose the 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. In order 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 about 20 kJ/L or 448 kJ/mol); in 'thermal' studies:

$$\Delta H = W + Q \qquad \text{(Eq 1)}$$

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 surrounds. 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. Equation (1) denotes 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 hydrolysed) 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 formalised as the 2nd Law of Thermodyamics:

$$\Delta H_{ATP} = \Delta G_{ATP} + T\Delta S_{ATP} \qquad \text{(Eq 2)}$$

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 non-zero velocity.) Using the Greek letter η to denote thermodynamic efficiency, we can now restate the 2nd Law expression of Eq 2:

$$\Delta H_{ATP} = W + (1 - \eta)\Delta G_{ATP} + T\Delta S_{ATP} = W + Q_{irrev} + Q_{rev} = W + Q \qquad \text{(Eq 3)}$$

in accord with the 1st 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*) while 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.

It is evident from Eq 3 that thermodynamic efficiency quantifies the proportion of the Gibbs Free Energy, inherent in ATP, that is utilised 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:

$$\varepsilon = W/\Delta H = W/(W + Q) \qquad \text{(Eq 4)}$$

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 zero 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:

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

Under suitable experimental conditions, designed such that basal metabolic rate can be discounted, then we speak of *Mechanical Efficiency*:

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

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 restoration of sarcolemmal gradients of Na^+ and K^+ and the sequestration of Ca^{2+} back into the sarcoplasmic reticulum (SR) following 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 % to 25 % of total cardiac metabolism. The brief flood of Ca^{2+} from the SR triggers the myosin-activated, ATP-dependent cycling of the actomyosin crossbridges 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: (a) 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 crossbridges (Eq 3), and (b) the entropy *exchanged* with the

surroundings through hydrolysis of ATP and which 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*:

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

There is sufficient creatine phosphate (CrP) in the myocytes to provide ATP via the Lohmann reaction to power the ionic pumps and crossbridges 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 CrP and ATP consumed during the initial (ionic and crossbridge) processes is labelled *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 in order to prevent both activation and cross-bridge cycling. In either case, basal enthalpy (ΔH_B) is found to be of comparable magnitude to that of Activation Enthalpy, comprising some 20 % to 25 % of Total Enthalpy (Gibbs and Loiselle, 2001).

We are now in a position to summarise the components of *Total Cardiac Enthalpy* production, which we do conceptually (Eq 7) and graphically (Figure3).

$$\Delta H_T = \Delta H_I + \Delta H_R + \Delta H_B \quad \text{(Eq 7)}$$

WHEREIN AND TO WHAT EXTENT COULD EFFICIENCY BE INCREASED?

We consider this question in four ways. Firstly, using the assumed proportions of each of the three components of Total Cardiac Enthalpy Production (Figure 3 and Eq 7), we sequentially set each to zero, assuming a starting value of Total Cardiac Efficiency of 0.20. This is an admittedly harsh approach since 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 unrealisable. Hence, secondly, we relax the constraints by allowing any non-zero 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 in order 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. Thirdly, 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 Figure 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 approximately 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 (ROS) (Chouchani *et al*., 2014) and 'electron leakage' – especially at Complexes I & 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 fibres 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 fibres). 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. In order 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 x 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 above), 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: (i) the rate of myocardial protein turnover is high, (ii) both degradation and synthesis require energy expenditure, and (iii) both the rate of basal heat

production and (iii) 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 ROS 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). 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 heart has been shown to be increased by both chronic and acute provision of palmitate (How *et al*., 2005; How *et al*., 2006), we nevertheless assume that Q_A is fixed at 0.2 ΔH_T, as shown in Figure 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: $\varepsilon_T \rightarrow 0.20/(1 - 0.25) = 0.27$

As is the case of basal heat production (above), the scope for improvement of Total Cardiac Efficiency by elimination of Activation Heat (without elimination of activation!) would be meagre.

(iv) Crossbridge heat ($Q_{X\text{-}b}$)

We assume that crossbridge heat arises primarily from inefficient conversion of the Gibbs Free Energy of ATP hydrolysis during crossbridge cycling. Such inefficiency is most readily visualised by reference to the formalism of Eisenberg and colleagues (1978; Eisenberg *et al*., 1980); we adopt the approach of those authors. An individual crossbridge 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 AF 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 Figure 3), then, at a relative afterload of 0.4, 40% is necessarily assigned to cross-bridge heat. If all crossbridge heat could be eliminated as a consequence of consuming Omega-3 fish-oils, then a 1.6-fold increase of cardiac efficiency would obtain.

- 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 crossbridges, *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 crossbridge work performance?

Barclay and co-workers (2010; Barclay, 2015), utilising the theoretical framework developed by Huxley and Simmons (1971, 1972, 1973), have inferred crossbridge 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 crossbridge 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 hydrolysed per 'crossbridge event', and by approximating the Gibbs Free Energy of a single ATP molecule to be 100 zJ (the quotient of 60 kJ/mole ATP and Avogadro's Number), they infer a crossbridge efficiency of 0.4. By contrast, crossbridges of cardiac muscle perform around 20 zJ per cycle. Thus, even if cardiac crossbridges 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 Equation 5: $\varepsilon_T = W/(W + Q_B + Q_A + Q_{X\text{-}b})$, sequentially setting each of the heat terms in the denominator to zero, in order 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 (Figure 3). In Figure 4A&B, we extend this analysis by allowing ε_1 to take on any value less than 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 (Figure 1).

c) Consideration of energetic components collectively and simultaneously

In both of the preceding analyses (Figure 4A&B), we have focussed 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 non-intervention ('control') group. We commence by noting that, in Figure 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) in order to achieve selected values of efficiency. From Equation 4:

$$\varepsilon_1 = W_1/(W_1 + Q_1) \text{ and } \varepsilon_2 = W_2/(W_2 + Q_2) \quad \text{(Eq 7)}$$

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

$$\frac{Q_2}{Q_1} = \frac{\varepsilon_1 * (1 - \varepsilon_2)}{\varepsilon_2 * (1 - \varepsilon_1)} \quad \text{(Eq 8)}$$

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 Figure 4C. They allow quantification of the extent to which heat output must diminish, in order 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 average efficiency of control hearts is initially 0.02 then, in order to increase it to 0.04 (at constant work-load), heat output would need to diminish by 50% (broken line segments). To increase it to 0.16 would require heat output to decrease by 90% (dotted blue lines). 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 insert - 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 2nd-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 Figure 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. These values were chosen to span regions of ε from near zero 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 Figure 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. In consequence, we are unable to reconcile our findings, that dietary fish-oils have no influence on cardiac efficiency (Figure 2), with reports in the literature (Figure 1, for example) to the contrary. In fact, from our consideration of thermodynamic constraints, we aver 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 Figure 4C. 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. We are at a loss to suggest 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 (and at the risk of allowing the algebra of Eq 8 to adumbrate the physiology that it models), the 'insert' of Figure 4C demonstrates that, by sufficient reduction of inefficiencies (i.e., heat production), perfect efficiency could be achieved by some intervention, independent of its value under 'control' conditions. Nevertheless, we remain puzzled by the published reports of striking improvements of total cardiac efficiency as a consequence of dietary enhancement with fish-oils and offer several putative attempts at reconciliation.

Attempts at Reconciliation

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 Figure 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 & Matsuoka (1915) used the blood-perfused heart-lung preparation to measure the total efficiency of the isolated dog heart, finding it 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 pre-load and after-load placed on the isolated working rat heart, while simultaneously measuring its rate of oxygen consumption, thereby allowing estimation of total cardiac efficiency. As pre-load was increased from 0 to 20 cm H_2O, efficiency increased from 4% to 17%. Note that 4% efficiency resulted from a pre-load of *zero*. 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 after-loaded contractions at room temperature. These authors reported an average peak value of total efficiency of 19% - in remarkable agreement with that arising from the aforementioned whole-heart study. Gibbs *et al.* could likewise observe very low values of efficiency (1.6 % to 5 %; see their Table 2) but *only* by increasing the after-load to the point where the muscle could scarcely shorten and work output was negligible. Such behaviour is implicit in the experimental data presented in Figure 2 and in the hypothetical situation presented in Figure 5. 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 under-estimation of peak efficiency of the ‘control group’.

2. Buffer-perfusion versus blood-perfusion.

This is the sole substantive difference of experimental protocols between our studies (as exemplified in Figure 2) and those of Pepe *et al.* (as exemplified in Figure 1). We rule out this difference as a likely contributor for four reasons. Firstly, 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 (Figure 1A). Secondly, any putative benefits of blood-perfusion were highly selective – benefiting efficiency in the presence of fish-oils but not in their absence. Thirdly, under even the harshest of perfusion protocols (periods of ischaemia followed by reperfusion), no difference was observed in either post-ischaemia, 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 forty years ago in the isolated rat heart that, despite variations of work and oxygen consumption, cardiac efficiency remained resolutely independent of haematocrit values ranging from 0% to 40%.

3. A ‘statistical’ artefact.

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 Figure 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 $0.25 \times 10^{-6.}$ This is such an improbably small value that we rule out the possibility of its occurrence. Thus, our puzzlement continues.

Post-Script

As indicated in the Introduction, our study was motivated, in part, by the publication of Fodor *et al.* which largely debunks the presumed scientific basis on which a diet rich in Omega-3 fish-oils is alleged to improve the health of the heart. We have since become equally disturbed by the publication of Ramsden *et al*.: *Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968-73)*, British Medical Journal 353: i1246, pages 1-17, 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) 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 a number of those 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.

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The authors declare no competing financial interests.

REFERENCES

Bang, H.O., and J. Dyerberg. 1972. Plasma lipids and lipoproteins in Greenlandic West Coast Eskimos. *Acta Med Scand*. 192:85-94.

Bang, H.O., J. Dyerberg, and N. Hjørne. 1976. The composition of food consumed by Greenland Eskimos. *Acta Med Scand*. 200:69-73.

Barclay, C.J. 1999. A weakly coupled version of the Huxley crossbridge model can simulate energetics of amphibian and mammalian skeletal muscle. *J Mus Res Cell Motil*. 20:163-176.

Barclay, C.J. 2015. Energetics of contraction. *In* Comprehensive Physiology. D.M. Pollock, editor. Wiley. 961-995.

Barclay, C.J., and C. Widén. 2010. Efficiency of cross-bridges and mitochondria in mouse cardiac muscle. *In* Muscle Biophysics: From Molecules to Cells, Advances in Experimental Medicine and Biology 682. D. Rassier, editor. Springer Science+Business Media LLC New York. 267-278.

Barclay, C.J., R.C. Woledge, and N.A. Curtin. 2010. Inferring crossbridge properties from skeletal muscle energetics. *Prog BiophysMol Biol*. 102:53-71.

Boardman, N.T., T.S. Larsen, D.L. Severson, M.F. Essop, and E. Aasum. 2011. Chronic and acute exposure of mouse hearts to fatty acids increases oxygen cost of excitation-contraction coupling. *Am J Physiol*. 300:H1631-H1636.

Brown, G.C. 1992. The leaks and slips of bioenergetic membranes. *FASEB Journal*. 6:2961-2965.

Chapman, B., and D.S. Loiselle. 2016. Thermodynamics and kinetics of the F_oF_1-ATPase: application of the Probability Isotherm. *Roy Soc Open Science*. 3.

Chapman, B., J. Mosse, and J. Larkins. 2011. The Probability Isotherm: an intuitive non-equilibrium thermodynamic framework for biochemical kinetics. *In* Proceedings of the Australian Conference on Science and Mathematics Education. M. Sharma, A. Yeung, T. Jenkins, E. Johnson, G. Rayner and J. West, editors. UniServe Science, the University of Sydney, Australia, University of Melbourne. 169-173.

Chouchani, E.T., V.R. Pell, E. Gaude, D. Aksentijevic, S.Y. Sundier, E.L. Robb, A. Logan, S.M. Nadtochiy, E.N.J. Ord, A.C. Smith, F. Eyassu, R. Shirley, C.-H.

Hu, A.J. Dare, A.M. James, S. Rogatti, R.C. Hartley, S. Eaton, A.S.H. Costa, P.S. Brookes, S.M. Davidson, M.R. Duchen, K. Saeb-Parsy, M.J. Shattock, A.J. Robinson, L.M. Work, C. Frezza, T. Krieg, and M.P. Murphy. 2014. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*. 515:431-435.

Divakaruni, A.S., and M.D. Brand. 2011. The regulation and physiology of mitochondrial proton leak. *Physiology*. 26:192-205.

Duvelleroy, M.A., M. Duruble, J.L. Martin, B. Teisseire, J. Droulez, and M. Cain. 1976. Blood-perfused working isolated rat heart. *J Applied Physiol* . 41:603-607.

Eisenberg, E., and T.L. Hill. 1978. A cross-bridge model of muscle contraction. *Prog Biophys Mol Biol* . 33:55-82.

Eisenberg, E., T.L. Hill, and Y.-D. Chen. 1980. Cross bridge model of muscle contraction: quantitative analysis. *Biophys J* . 29:195-227.

Evans, C.L., and Y. Matsuoka. 1915. The effect of various mechanical conditions on the gaseous metabolism and efficiency of the mammalian heart. *J Physiol*. 49:378-405.

Fodor, J.G., E. Helis, N. Yazdekhasti, and B. Vohnout. 2014. “Fishing” for the Origins of the “Eskimos and Heart Disease” Story: Facts or Wishful Thinking? *CanJ Cardiol*. 30:864-868.

Galiñanes, M., P. Bernocchi, V. Argano, A. Cargnoni, R. Ferrari, and D.J. Hearse. 1996. Dichotomy in the post-ischemic metabolic and functional recovery profiles of isolated blood- versus buffer-perfused heart. *J Mol Cell Cardiol*. 28:531-539.

Gibbs, C.L. 1978. Cardiac energetics. *Physiol Rev*. 58:174-254.

Gibbs, C.L., and C.J. Barclay. 1995. Cardiac efficiency. *CardiovascRes*. 30:627-634.

Gibbs, C.L., and D.S. Loiselle. 2001. Cardiac basal metabolism. *Jap J Physiol* 51:399-426.

Gibbs, C.L., W.F.H.M. Mommaerts, and N.V. Ricchiuti. 1967. Energetics of cardiac contractions. *J. Physiol.* 191:25-46.

Goo, S., J.-C. Han, L.A. Nisbet, I.J. LeGrice, A.J. Taberner, and D.S. Loiselle. 2014a. Dietary pre-exposure of rats to fish oil does not enhance myocardial efficiency of isolated working-hearts or their left ventricular trabeculae. *J Physiol*. 592:1795-1808.

Goo, S., J.-C. Han, L.A. Nisbet, I.J. LeGrice, A.J. Taberner, and D.S. Loiselle. 2014b. Dietary supplementation with either saturated or unsaturated fatty acids does not affect the mechanoenergetics of the isolated rat heart. *Physiol Reports*. 2.

Goo, S., T. Pham, J.-C. Han, P. Nielsen, A. Taberner, A. Hickey, and D. Loiselle. 2013. Multiscale measurement of cardiac energetics. *Clinical and ExpPharmacol Physiol*. 44:49-62.

Hartree, W., and A.V. Hill. 1922. The recovery heat-production in muscle. *The J Physiology*. 56:367-381.

Herbst, E.A.F., S. Paglialunga, C. Gerling, J. Whitfield, K. Mukai, A. Chabowski, G.J.F. Heigenhauser, L.L. Spriet, and G.P. Holloway. 2014. Omega-3 supplementation alters mitochondrial membrane composition and respiration kinetics in human skeletal muscle. *J Physiol*. 592:1341-1352.

Hill, A.V. 1949. The heat of activation and the heat of shortening in a muscle twitch. *Proc Royal Soc B*. 136:195-211.

How, O.-J., E. Aasum, S. Kunnathu, D.L. Severson, E.S.P. Myhre, and T.S. Larsen. 2005. Influence of substrate supply on cardiac efficiency, as measured by pressure-volume analysis in ex vivo mouse hearts. *Am J Physiol*. 288:H2979-2985.

How, O.-J., E. Aasum, D.L. Severson, W.Y.A. Chan, M.F. Essop, and T.S. Larsen. 2006. Increased myocardial oxygen consumption reduces cardiac efficiency in diabetic mice. *Diabetes*. 55:466-473.

Huxley, A.F. 1957. Muscle structure and theories of contraction. *Progress in Biophys BiophysChem*. 7:255-318.

Huxley, A.F., and R.M. Simmons. 1971. Proposed mechanism of force generation in striated muscle. *Nature*. 233:533-538.

Huxley, A.F., and R.M. Simmons. 1972. Comments on the mechanism of force generation in striated muscles. *Nature*. 239:183-187.

Huxley, A.F., and R.M. Simmons. 1973. Mechanical transients and the origin of muscular force. *In* Cold Spring Harbour Symposium on Quantitative Biology. Cold Spring Harbour. 669-680.

Jastroch, M., A.S. Divakaruni, S. Mookerjee, J.R. Treberg, and M.D. Brand. 2010. Motochondrial proton and electron leaks. *Essays Biochem*. 47:53-67.

Loiselle, D.S. 1987. Cardiac basal and activation metabolism. *Bas Res Cardiol*. 82 (Suppl 2):37-50.

Lou, F., W.J. van der Laarse, N.A. Curtin, and R.C. Woledge. 2000. Heat production and oxygen consumption during metabolic recovery of white muscle fibres from the dogfish *Scyliorhinus canicula*. *J ExpBiol*. 203:1201-1210.

McLennan, P.L., M.Y. Abeywardena, J.A. Dallimore, and D. Raederstorff. 2012. Dietary fish oil preserves cardiac function in the hypertrophied rat heart. *Bri J Nutrit*. 108:645-654.

Neely, J.R., H. Liebermeister, E.J. Battersby, and H.E. Morgan. 1967. Effect of pressure development on oxygen consumption by isolated rat heart. *Am J Physiol*. 212:804-814.

Peoples, G.E., P. Howe, P.L. McLennan, and H. Groeller. 2008. Fish oil reduces heart rate and oxygen consumption during exercise. *J Cardiovasc Pharmacol*. 52:540-547.

Pepe, S., and P.L. McLennan. 2002. Cardiac membrane fatty acid composition modulates myocardial oxygen consumption and postischemic recovery of contractile function. *Circ*. 105:2303-2308.

Pepe, S., and P.L. McLennan. 2007. (n-3) Long chain PUFA dose-dependently increase oxygen utilization efficiency and inhibit arrhythmias after saturated fat feeding in rats. *J Nutrit*. 137:2377-2383.

Pham, T., D. Loiselle, A. Power, and A.J.R. Hickey. 2014. Mitochondrial inefficiencies and anoxic ATP hydrolysis capacities in diabetic rat heart. *Am J Physiol*. 307:C499-C507.

Power, A., T. Pham, N. Pearson, C. Cheung, A.R.J. Phillips, and A.J.R. Hickey. 2014. Uncoupling of oxidative phosphorylation and ATP synthase reversal within the hyperthermic heart. *Physiol Reports*. 2:e12138.

Sandhu, R., R.J. Diaz, and G.J. Wilson. 1993. Comparison of ischaemic preconditioning in blood perfused and buffer perfused isolated heart model. *Cardiovascr Res*. 27:602-607.

Yu, L., B.D. Fink, J.A. Herlein, C.L. Oltman, K.G. Lamping, and W.I. Sivitz. 2014. Dietary fat, fatty acid saturation and mitochondrial bioenergetics. *J Bioenerg Biomemb*. 46:33-44.

FIGURE LEGENDS

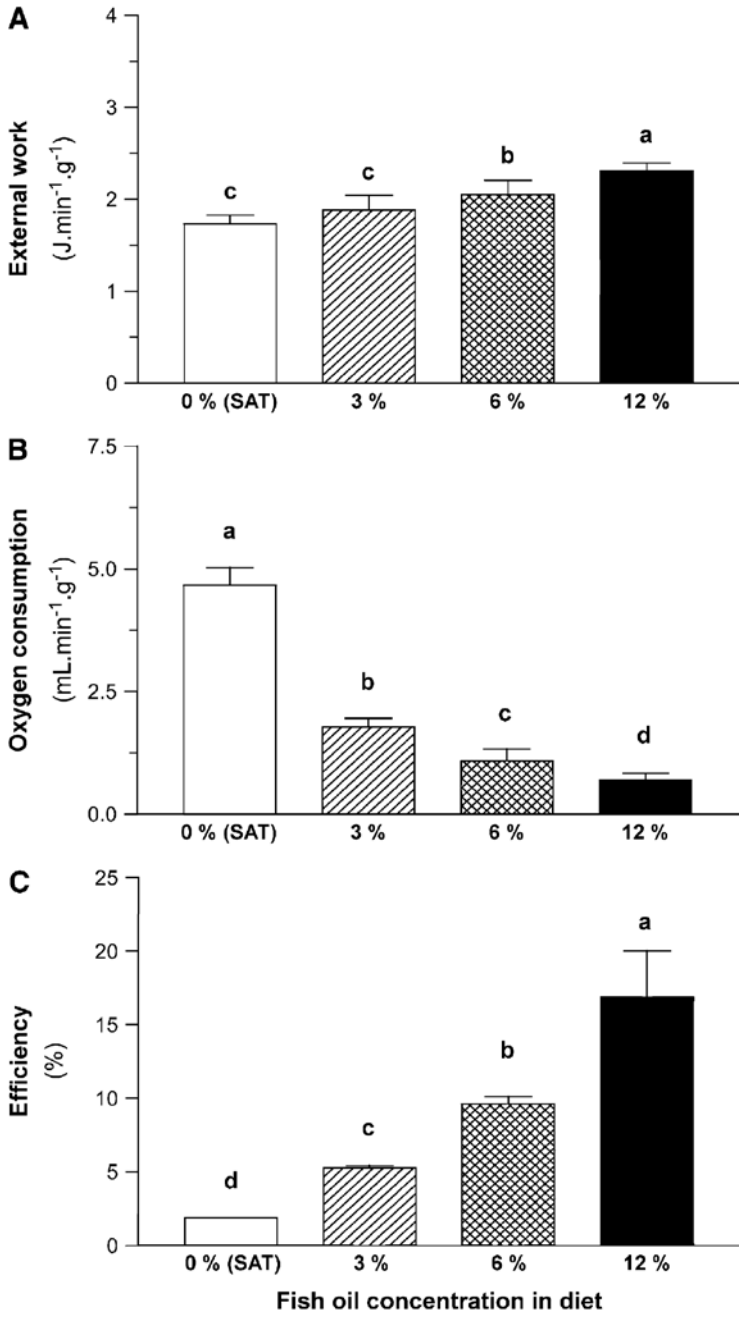

Figure 1. External work (*A*), myocardial oxygen consumption (*B*), and 'cardiac energy utilization efficiency' (C) during normoxic baseline function of the erythrocyte-perfused, isolated working hearts of rats that consumed the SAT- or FO-supplemented diets for 6 wk. Values are means ± SD, $n = 10$. Bars without a common letter differ, $P < 0.05$. [Reproduced from Figure 1 of Pepe & McLennan, with permission of The Journal of Nutrition 137: 2377-2383, 2007, via Rights Link.]

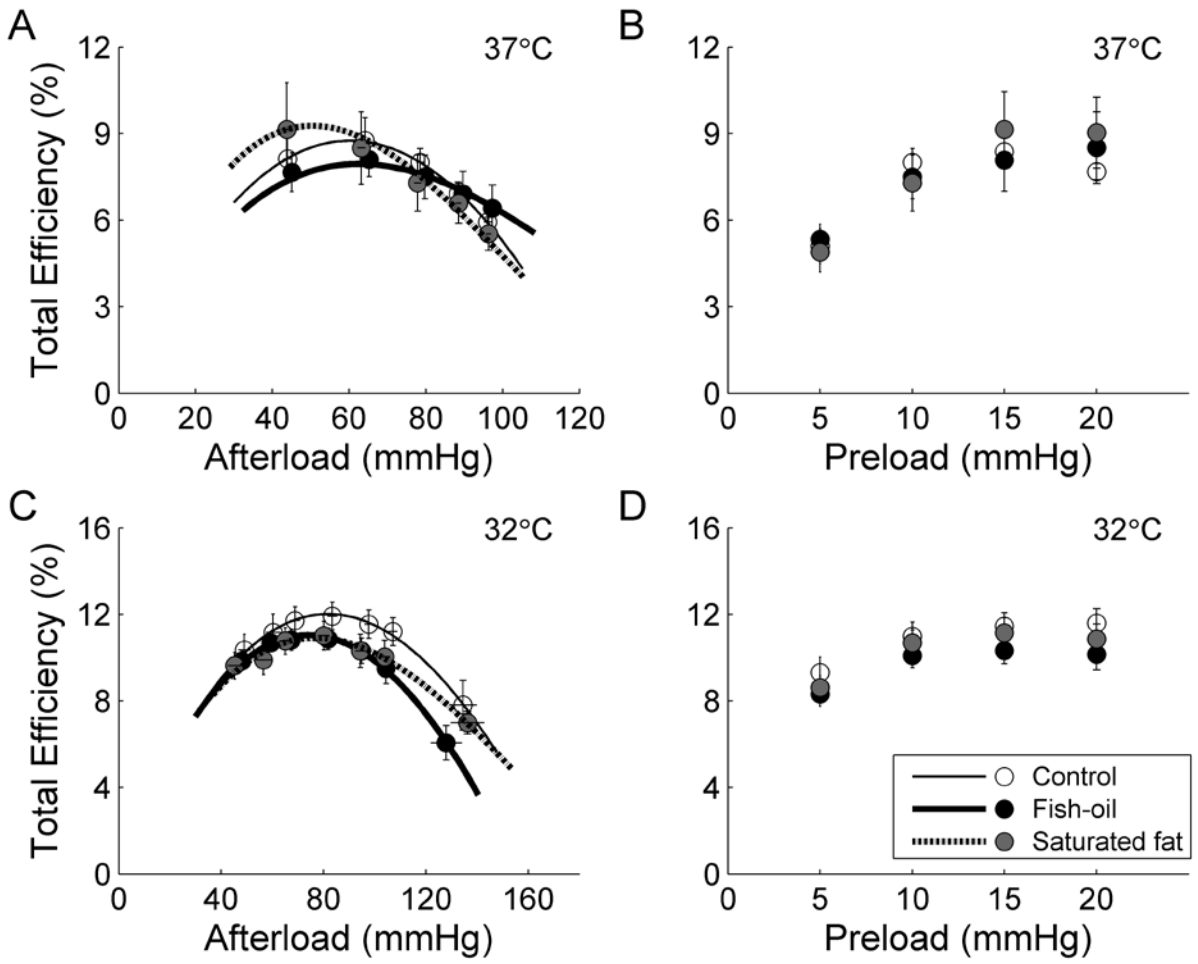

Figure 2. Total efficiency (W/ΔH) of isolated, saline-perfused rat hearts at 37 °C (A & B) and 32 °C (C & D) from rats fed isocaloric diets. W = pressure-volume work; ΔH = molar enthalpy of oxygen. **A & C**: effect of variable afterload at a fixed pre-load of 10 mmHg. **B & D**: effect of variable pre-load at a fixed afterload of 75 mmHg. No differences among diets in any panel. [Reproduced from Figure 2, Goo *et al*., (2014a) and Figures 4&5, Goo *et al*. (2014b), under the Rights of Authors of APS Articles and Physiology Reports, respectively.]

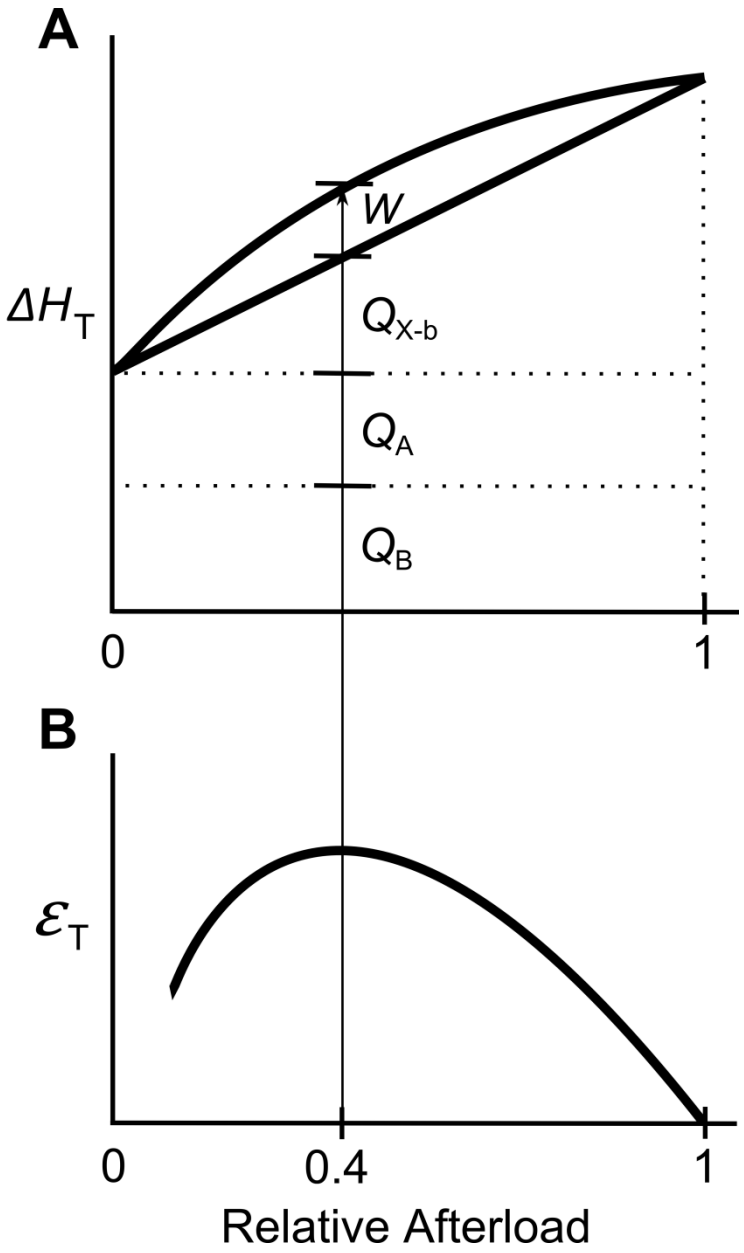

Figure 3. Schematic diagram of the components of total enthalpy expenditure (ΔH_T), work (W), cross-bridge heat ($Q_{x\text{-}b}$), activation heat (Q_A) and basal heat (Q_B), Panel **A**, and total efficiency (ε_T), Panel **B**, as functions of relative afterload. The vertical line at 0.4 relative afterload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

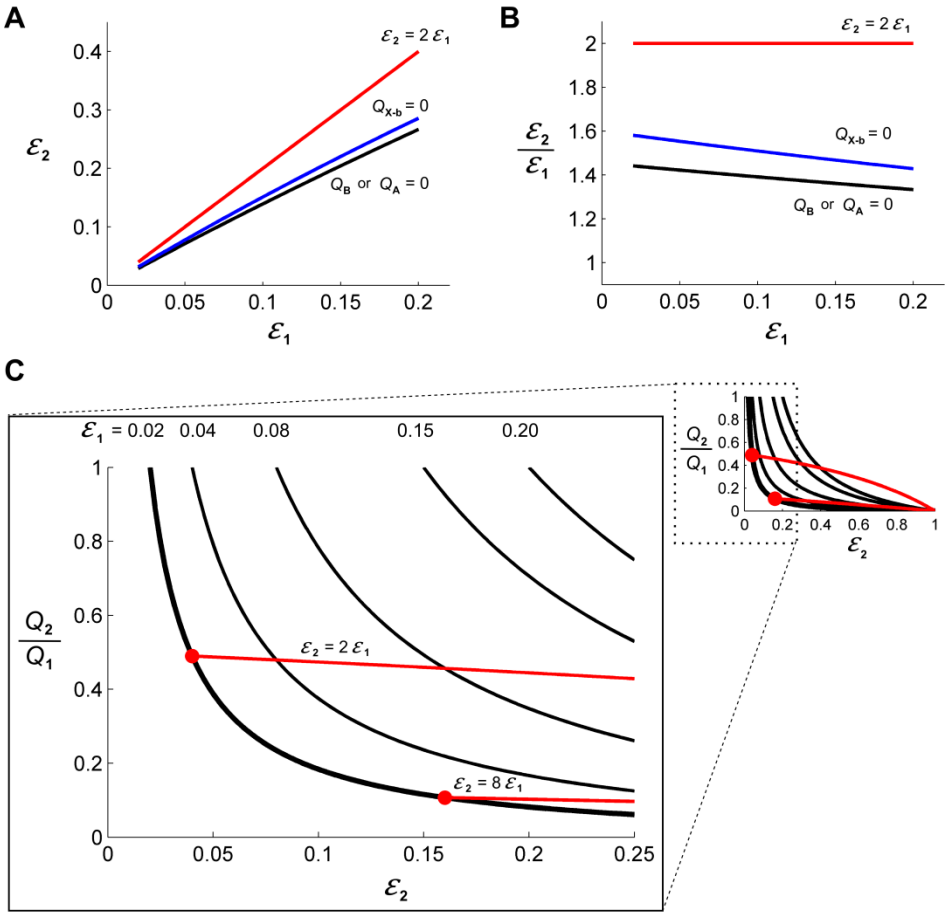

Figure 4. Expected results from setting, independently, each of individual thermal components to zero (Panels A & B), and from considering, collectively, all thermal components simultaneously (Panel C). In Panels A&B, black lines denote, respectively, the absolute (ε_2) or relative ($\varepsilon_2/\varepsilon_1$) improvements of efficiency in an 'Intervention' group with respect to its 'Control' group by setting either Q_B or Q_A to zero and the blue line by setting $Q_{X\text{-}b}$ to zero. The red lines denote the extent of improvement required in order to achieve a doubling of efficiency (mimicking the minimal increment of efficiency shown in Figure 1C. In Panel **C**, the black lines (at ε_1 = 0.02, 0.04, 0.08, 0.15 and 0.20) denote the extent to which heat output must be reduced in order for an intervention to increase total cardiac efficiency to ε_2 at fixed work-load. The red lines show that, commencing from $\varepsilon_1 = 0.02$ (corresponding to the Control Group in Figure 1), heat output would need to reduce by 50% in order to double efficiency and by 90% in order to increase it 8-fold in order to mimic the results shown in Figure 1C. The boxed region of the insert encloses the range of estimates of total cardiac efficiency typically reported in the Literature.

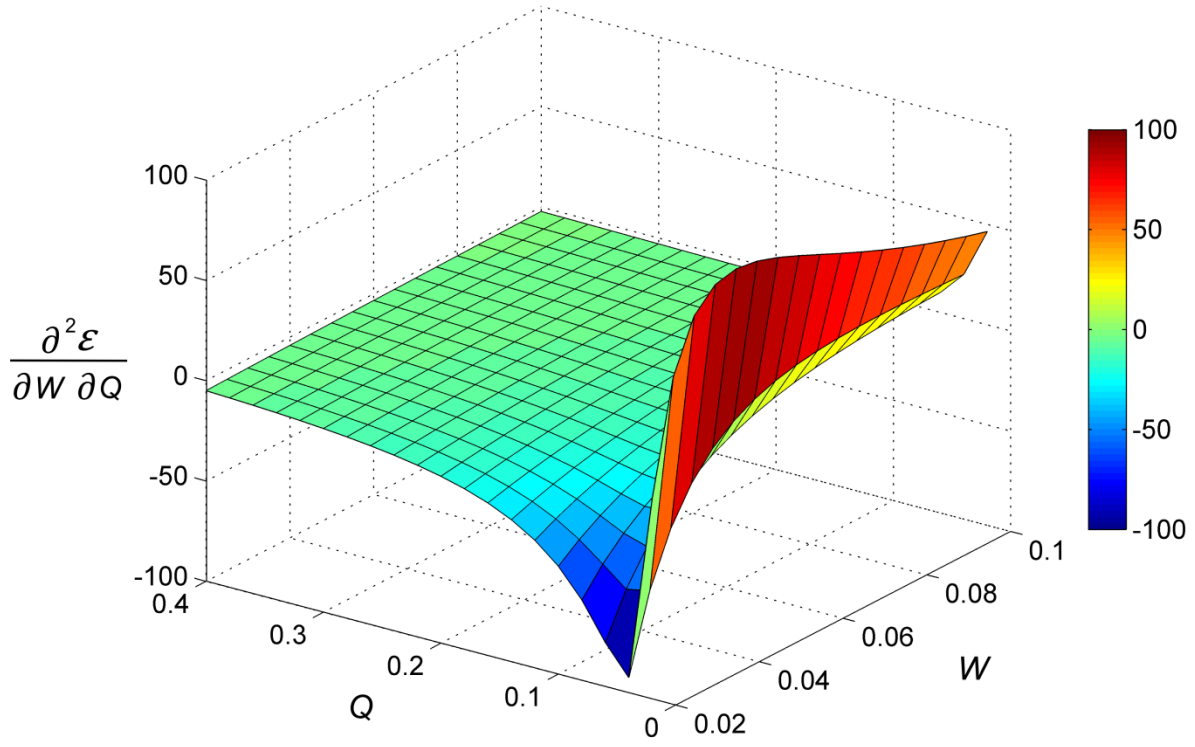

Figure 5. Three-dimensional surface plot showing the consequences, to calculated values of Total Cardiac Efficiency (ε), of errors of measurement in estimating work (W) and heat (Q).

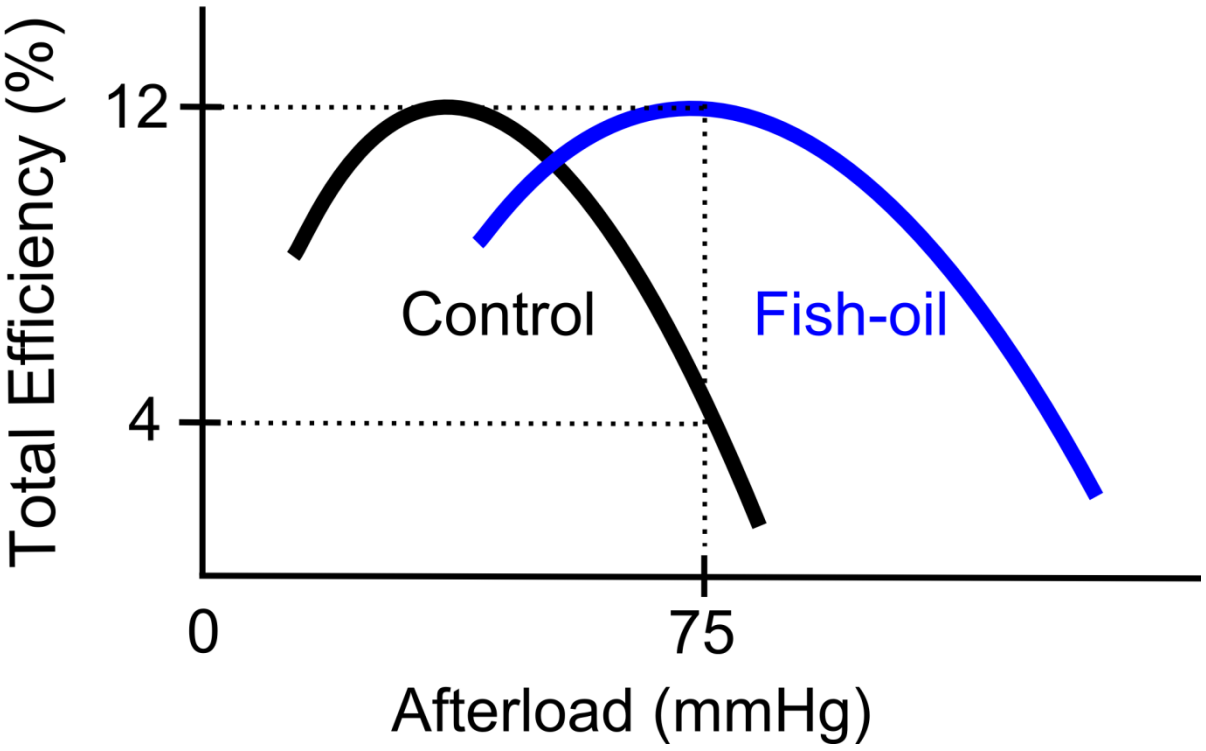

Figure 6. Schematic comparison of differently located Efficiency-Afterload relations. Note the identical afterloads and the identical *true* efficiencies (greatly underestimated for the Control group).