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Microcalorimetric study of myocardial tissue metabolism in experimental hyperthyroidism in rats

Stig Valdemarsson [a,*], Mario Monti [b]

[a] *Department of Internal Medicine, Lund University Hospital, S-221 85, Lund, Sweden*
[b] *Department of Cell Biology, Lund University Hospital, S-22185, Lund, Sweden*

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Abstract

The metabolic consequences in myocardial tissue, specifically concerning the importance of aerobic metabolism, following the stimulatory actions of thyroid hormones were studied in an experimental model for hyperthyroidism in rats, treated with triiodothyronine (T3) for two weeks. The metabolic activity in myocardial tissue was measured by microcalorimetry and related to oxygen consumption and to the ATP content.

In myocardial muscle tissue from rats given 10–25 μg T3 daily over two weeks, heat production was $1.53 \pm 0.09\,mW\,g^{-1}$, compared to $0.94 \pm 0.05\,mW\,g^{-1}$ in controls, $p < 0.001$. Similarly, oxygen consumption was higher in T3-treated rats, $2.75 \pm 0.16\,nmol\ O_2\ s^{-1}\,g^{-1}$, compared to $1.55 \pm 0.14\,nmol\ O_2\ s^{-1}\,g^{-1}$ in the controls, $p < 0.001$. There was a significant correlation between heat production and oxygen consumption values when data from controls and T3-treated rats were put together, $r = 0.66$, $p < 0.001$. The ATP content in the myocardial tissue was significantly lower in T3-treated rats than in control rats, 2.82 ± 0.53 and $7.91 \pm 0.44\,\mu mol\,g^{-1}$ respectively, $p < 0.001$. The myocardial tissue ATP content was inversely correlated to myocardial heat production, $r = -0.54$, $p < 0.01$, and oxygen consumption, $r = -0.50$, $p < 0.05$.

In conclusion, the present data demonstrate the close relationship between oxygen consumption and metabolic activity in rat myocardial tissue in the euthyroid and hyperthyroid states. Aerobic metabolism was calculated to account for about 80% of the myocardial heat production in the euthyroid state and for about 87% in the hyperthyroid state. In addition, T3 treatment was associated with a significant reduction of the ATP content in the myocardial tissue studied, suggesting that myocardial ATP generation may be insufficient to meet the increased demand on heart performance in hyperthyroidism.

Keywords: Aerobic metabolism; Anaerobic metabolism; Cell metabolism; Hyperthyroidism; Myocardial metabolism; Microcalorimetry; Thyroid hormones

* Corresponding author. Fax: Int +46(0)46 2110908.

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1. Introduction

Hyperthyroidism is well known to be associated with alterations in myocardial performance including positive chronotropic action, as well as enhanced left ventricular end-diastolic volume and left ventricular contractility [1]. The effect of thyroid hormone excess on myocardial function has been proposed to be exerted directly through thyroid hormone nuclear receptors and also via enhanced adrenoceptor sensitivity [2]. DNA binding triiodothyronine (T3) receptor proteins have been identified in mammalian myocardium [3]. Thyroid hormones may thus regulate the gene expression of certain cardiac proteins like the sarcoplasmatic reticulum calcium-activated ATPase, influencing the intracellular calcium level of the myocardial cell and thereby the systolic tension and diastolic relaxation [4]. In addition to these direct effects on heart cells, thyroid hormone excess will also affect myocardial work through their effects on the function of the systemic vascular system and other organs as well [5, 6].

To meet the increased demand on heart performance during thyroid hormone excess, myocardial energy consumption is enhanced [7]. Thyroid hormones stimulate aerobic metabolism in myocardial cells in vivo [8, 9]. The role of anaerobic metabolism for the increased expenditure of energy in cardiomyocytes in hyperthyroidism is less well known. In lymphocytes, however, anaerobic metabolism has been found to be of great importance for the increased energy expenditure in the hyperthyroid state [10].

An enhanced generation of adenosin tri-phosphate (ATP) is essential for the cells to meet the stimulatory effects of thyroid hormone excess on myocardial performance. A defective regulation of mitochondrial ATP synthesis in heart cells from rats given thyroxin has, however, been noted [11]. This latter finding might suggest that the ATP content in myocardial tissue could be a limiting factor in myocardial cell energy expenditure and function in hyperthyroidism. To investigate this possibility further, we induced a hyperthyroid state in rats by triiodthyronine administration and then studied the metabolic activity of myocardial tissue by microcalorimetry and related the calorimetric data to the ATP content and oxygen consumption of the heart tissue.

2. Material and methods

2.1. Animals and preparational processes:

Male Wistar rats, about 200 g b.w., were used for controls and in the T3 treatment experiments. They were kept in separate cages with a regular day-night regimen and with free access to food and drinking water.

In one group of 15 rats, hyperthyroidism was induced by daily subcutaneous injections of 10 ($n = 8$) or 25 ($n = 7$) μg triidothyronine (Liothyronine, Nycomed, Sweden) for 2 weeks, with the last injection given 2 h before sacrifice. A clearly increased level of free T3 (> 20 pmol l^{-1} two hours after injection) in blood collected at the sacrifice was documented in some of the animals to assure appropriate resorption of the drug. The free thyroxine (T4) level was below the detection limit in the same samples studied.

Twenty-three rats were kept under similar conditions and received daily injections of saline, the amount being the same as in the T3-treated group. These rats served as controls.

In order to avoid any inhibiting influence of sedatives on metabolic processes, the animals were sacrificed by decapitation. The heart was immediately prepared and tissue slices were cut from the apical region for further preparation for microcalorimetry and oxygen consumption measurements, about 5 mg each. A separate specimen was immediately frozen in liquid nitrogen for later ATP analysis.

The tissue slices for microcalorimetry and oxygen consumption studies were carefully handled and kept until measurement at 37°C in Krebs–Ringer solution (values in mol dm^{-3}): 0.12 NaCl, 0.015 $NaHCO_3$, 0.05 KCl, 0.015 $CaCl_2$, 0.012 Na_2HPO_4, supplemented with 0.00825 glucose and insulin (Actrapid, Novo Nordisk, Denmark) at a concentration of 0.1 Unit cm^{-3} buffer.

The study was approved by the Local Ethical Committee of the University of Lund.

2.2. *Microcalorimetric and oxygen consumption measurements*

The heat production rate of the myocardial tissue sample was measured by a microcalorimeter of the thermopile heat conduction type [12], arranged as a twin instrument with one of the units containing the reactive system and the other containing the reference ampoule with water. Measurements were performed at 37°C. A 3.0 or 0.7 ml flow-through vessel for liquid perfusion was used. A good baseline stability was obtained for 12 h, established with the vessel charged with water.

The voltage output was amplified 1000 times with a Keithley 150 B Microvoltmeter, 10 μV range. Calibration was carried out electrically between the experiments. The calorimetric vessel was thermostated at four consecutive heat exchange positions before reaching the thermopile zone after 15–20 min. The tissue slice was kept static on a thin stainless steel needle inside the steel ampoule during the experiment. The perfusion medium was introduced very close to the muscle sample by the use of a peristaltic pump (LKB Perpex, Sweden) in order to keep a constant pH during the measurement procedure; perfusion rate was 5 $cm^3\ h^{-1}$. Carbogen (95% O_2 + 5% CO_2) was bubbled through the perfusion medium outside the calorimeter. Calorimetric recordings lasted several hours. During the registration period, the pH in the effluent was measured intermittently by a radiometer type G 297/G7 capillary electrode; only a very slight pH change was detected, from 7.8 to 7.3.

The heat production rate, as calculated from the power–time curves, refers to 2 h after the start of the calorimetric experiment, and is expressed in units of mW g^{-1} of tissue. The coefficient of variation for the method is estimated to be 4.2% [13].

For oxygen consumption studies, the myocardial slice was put in a 1-ml measurement chamber of a Clark oxygen electrode (Hansatech, UK) where the rate of oxygen consumption could be continuously monitored at 37°C, expressed as nmol O_2 uptake $s^{-1}\ g^{-1}$ wet tissue. The ATP content was determined by a luminometric method (Luminometer 1251, LKB-Wallac, Turku, Finland) according to an earlier description [14].

2.3. Statistics

Data are expressed as mean ± SE. Differences between means were calculated by Student's *t*-test. Correlations between variables were calculated by the method of least squares.

3. Results

3.1. Myocardial cell metabolism

In the T3-treated group, heat production (P) was significantly higher compared to the controls; 1.53 ± 0.09 (mean ± SE) $mW\,g^{-1}$ ($n = 15$) and $0.94 \pm 0.05\,mW\,g^{-1}$ ($n = 23$), respectively, $p < 0.001$ (Fig. 1).

Similarly, oxygen consumption (V_{O_2}) was significantly higher in T3-treated rats ($n = 15$), 2.75 ± 0.16 nmol $O_2\,s^{-1}\,g^{-1}$, compared to 1.55 ± 0.14 nmol $O_2\,s^{-1}\,g^{-1}$ in the controls ($n = 20$), $p < 0.001$ (Fig. 1).

There was no significant difference with regard to heat production or oxygen consumption between the groups of rats given 10 or 25 μg T3 daily.

In the controls ($n = 20$), a significant correlation was found between heat production and oxygen consumption in myocardial tissue, $r = 0.49$, $p < 0.05$. When this relationship between the heart P and V_{O_2} values was calculated for the controls and the T3-treated groups together ($n = 35$), the coefficient of correlation was 0.66, $p < 0.001$, Fig. 2.

3.2. ATP content

The heart ATP content in control rats ($n = 9$) was $7.91 \pm 0.44\,\mu mol\,g^{-1}$ but significantly lower in the whole group of rats given T3 ($n = 15$), $2.82 \pm 0.53\,\mu mol\,g^{-1}$, $p < 0.001$. In rats given 10 or 25 μg of T3 daily, the myocardial tissue of ATP content was 3.81 ± 0.94 and $1.83 \pm 0.14\,\mu mol\,g^{-1}$, respectively, Fig. 3.

The myocardial tissue ATP content was inversely correlated to myocardial heat production, $r = -0.54$, $p < 0.01$, Fig. 4A, and to myocardial oxygen consumption, $r = -0.50$, $p < 0.05$, Fig. 4B.

4. Discussion

The main findings in the present study was the demonstration that triiodothyronine (T3) treatment over two weeks is followed by an increased heat production in myocardial tissue that is closely associated with an enhanced oxygen consumption, and also with a significant reduction in the ATP content of the heart tissue. As expected, oxygen consumption increased at a similar magnitude as the increase in heat production. Assuming that glucose, the only energy substrate present in the buffer medium, was used by the myocardial cells, then aerobic metabolism in the myocardial tissue

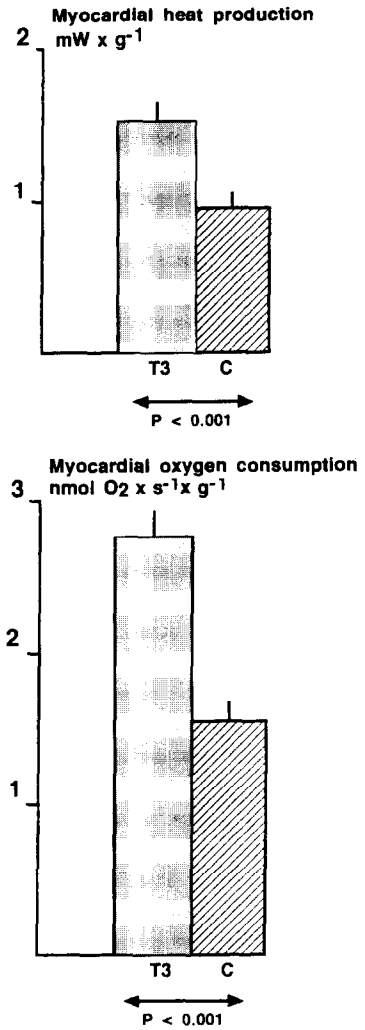

Fig. 1. Heat production and oxygen consumption in myocardial muscle in rats treated with triiodothyronine (T3) or saline (C) for 2 weeks.

would account for about 80% of the myocardial heat production in the controls and for about 87% in the T3-treated rats. This strongly indicates that aerobic metabolism is of major importance for the metabolic activity in the rat heart in the euthyroid state as well as in the hyperthyroid state. However, energy production along anaerobic metabolic pathways seems to contribute to a minor degree of myocardial energy production. The relative contribution from anaerobic metabolism was, however, not further increased in the hyperthyroid state. A significant correlation between heat production and oxygen consumption rates in myocardial muscle tissue in controls, and also when data from controls and T3-treated rats were put together, further supports

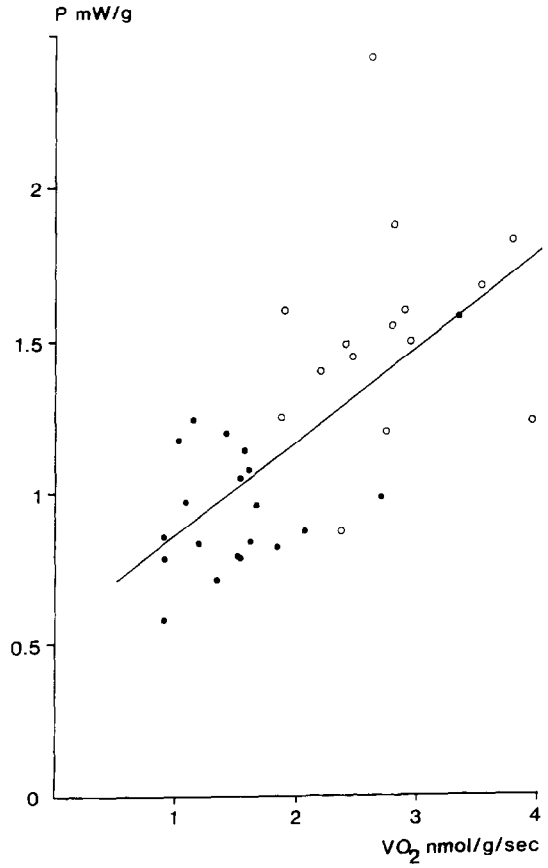

Fig. 2. Relationship between heat production (P) and oxygen consumption (V_{O_2}) in myocardial muscle tissue from rats given daily injections of triiodothyronine (○) or saline (●) for 2 weeks: $r = 0.66$, $p < 0.001$.

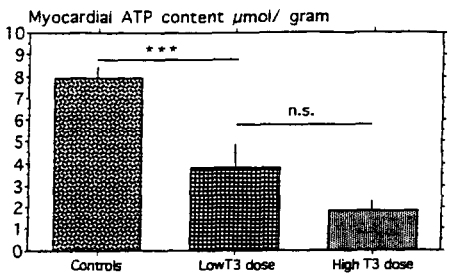

Fig. 3. The ATP content in myocardial tissue from rats given saline (controls, $n = 9$) or a low (10 µg, $n = 8$) or a high (25 µg, $n = 7$) dose of triiodothyronine once daily for two weeks.

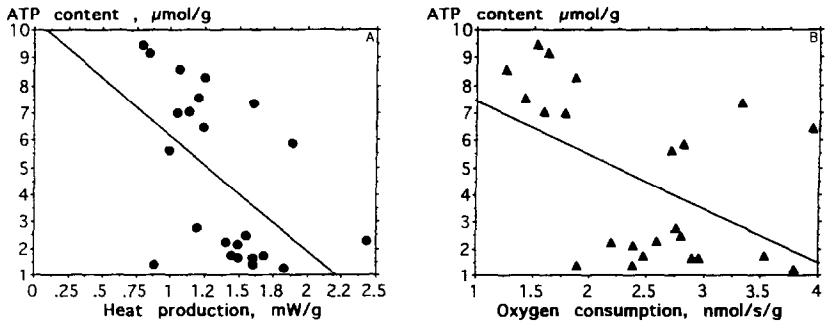

Fig. 4. Myocardial content of ATP in relation to heat production (A, $r = -0.54$, $p < 0.01$) and oxygen consumption (B, $r = -0.50$, $p < 0.05$) in myocardial tissue from rats. The relationship was calculated from data from control animals together with data from rats who had been treated with triiodothyronine for two weeks to induce a hyperthyroid state.

a close relationship between total cell metabolic activity and oxygen consumption in the rat heart. This is in agreement with earlier observations of a linear relationship between the increased rate of oxygen uptake in the hyperthyroid heart and expressions of heart work like pulse rate and cardiac index [8].

Oxygen consumption studies have been used to evaluate aerobic metabolism but will not, however, reflect the complete metabolic situation of the cells, since anaerobic metabolism is not included [9]. Microcalorimetry has been found to be suitable for studies of muscle metabolism [15–18]. The heat production will reflect directly the total metabolic activity of the tissue sample, taking into account aerobic as well as anaerobic metabolism. According to the present microcalorimetric and oxygen consumption studies, a minor anaerobic contribution to rat myocardial energy expenditure seems to be present.

Studies of the effects of thyroid hormone at the cell level have advanced with the clarification of the action of T3 at specific nuclear receptors. Of the T3 binding subtypes of thyroid hormone receptors, the β_1-receptor has been found in thyroid-hormone-sensitive tissues, including rat heart [19]. This forms the basis for a stimulatory effect of T3 on the expression of proteins of importance for cardiac contractility. Thus T3 may cause an enlargement of the calcium transporting activity and capacity in the sarcoplasmic reticulum [7], induce a shift towards a more rapidly contracting α-myosin isoform [20], change Na^+/K^+-ATPase isoforms [2], and affect atrial natriuretic factor [21] and cytosolic malic enzyme [22]. Furthermore, thyroid hormone may also exert extranuclear and plasma membrane effects.

The consequence of these stimulatory effects of T3 will be an enhanced energy consumption, supplied by increased ATP generation. However, in the present study, we found a profound decrease in the myocardial tissue ATP content in T3-treated rats. This could be due either to a defect ATP generation or to an increased ATP expenditure, or to a combination of these possibilities in the hyperthyroid state. Decreased myocardial ATP content has previously been noted in spontaneously hypertensive rats given T3 [23], and also during electrical stimulation of car-

diomyocytes from thyroxine-treated rats [11] and, although more slowly developed, in soleus muscle from T3-treated rats [24]. A defective regulation of mitochondrial ATP synthesis in heart cells has been suggested in hyperthyroid rats [11]. The results of the present study are in accordance with these previous findings, showing a decreased ATP content in the rat myocardial tissue during exposure to high levels of triiodothyronine. In the present investigation we found inverse relationships between the ATP content on the one hand, and oxygen consumption and heat production in heart tissue on the other. Thus, an enhanced use of oxygen by the myocardial tissue was not followed by increasing levels of ATP content in the tissue sample studied. This could be explained by an increased ATP consumption following the influence of thyroid hormones on cell function. This idea is supported by the inverse relationship found between the heat production and the ATP content of the myocardial tissue, indicating that the hyperthyroid state is associated with a high ATP expenditure. These data suggest that the ATP supply could be a limiting factor for further energy expenditure, and is thus possibly insufficient to meet the increased demand on myocardial performance induced by thyroid hormone stimulation.

The data from the present investigation demonstrate that myocardial metabolism is affected after only two weeks of T3 treatment. Whether the observed effects are due to direct nuclear effects of T3 or to an enhanced adrenoceptor sensitivity, or to a combination of these mechanisms, cannot be evaluated from our present study. In lymphocytes from hyperthyroid patients, we found an increased heat production without a corresponding elevation of lymphocyte oxygen consumption, suggesting that the thyroid-hormone-dependent increase of energy expenditure in these cells is due to anaerobic metabolism [10]. Treatment with β-adrenoceptor antagonists is associated with normal lymphocyte metabolism, suggesting that the increased metabolic activity in lymphocytes during thyroid hormone excess is due to an enhanced adrenoceptor activity [25]. According to the present study, myocardial tissue differs from lymphocytes, showing a more close relationship between aerobic metabolism and energy expenditure, not only in the euthyroid but also in the hyperthyroid state. Most probably, an enhanced adrenoceptor sensitivity as well as nuclear thyroid hormone effects contribute to the increased myocardial heat production after T3 administration. A specific sensitivity of the human heart to thyroid hormones with regards to average 24-h heart rate, atrial premature beats, left ventricular mass and cardiac contractility has recently been reported during suppressive thyroxin substitution therapy [26].

In conclusion, present data have extended our knowledge on the action of thyroid hormones and heart function by demonstrating the close relationship between oxygen consumption and the increased metabolic activity taking place in rat myocardial tissue, although a role for anaerobic metabolism should be considered. Moreover, there was a significant reduction of the ATP content in rat myocardial tissue and an inverse relationship between the ATP content, on the one hand, and oxygen consumption and heat production in rat myocardial tissue on the other, suggesting that myocardial ATP generation might be insufficient to meet the increased demand on heart performance in hyperthyroidism.

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