Biochemical analysis of muscle biopsy in overnight fasting patients with severe chronic heart failure

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To better characterize the role of skeletal muscle in chronic heart failure we studied energetic charge, metabolites and enzyme activity in the energy production pathway.

We selected 15 males with severe chronic heart failure (NYHA class III, stable clinical conditions and in normal nutritional status) and seven controls. Controls and patients were submitted to biopsy of the vastus lateralis muscle in resting and fasting conditions. Hormone profiles were also evaluated.

Our results showed near normal ATP, ADP and AMP concentrations, but there were substantially more reductions in glycogen (46 ± 5 vs 77 ± 6 umoles glycosidic units . g⁻¹ fresh tissue) and creatine phosphate (5 ± 1 vs 13 ± 1 umoles . g⁻¹ fresh tissue) in patients than in controls. We also found a reduction in glycolytic activity (pyruvate kinase 1009 ± 79 vs 1625 ± 26 nmoles . min⁻¹ . mg protein⁻¹), despite normal tricarboxylic acid cycle velocity, an increase in alanine aminotransferase (964 ± 79 vs 425 ± 34 nmoles . min⁻¹ . mg protein⁻¹) and in aspartate aminotransferase (515 ± 44 vs 291 ± 56 nmoles . min⁻¹ . mg protein⁻¹). An increase was also observed in total NADH cytochrome c reductase (128 ± 14 vs 68 ± 5 nmoles . min⁻¹ . mg protein⁻¹), while cytochrome oxidase activity was normal. The cortisol/insulin ratio was slightly elevated (77 ± 4 vs 32 ± 12).

In conclusion, normonutritive patients with severe heart failure show an imbalance in the energy production/utilization ratio. The impairment is probably due both to a decrease in production and an increase in consumption of energy owing to greater cellular workload and/or a hypercatabolic state.

Key Words: Heart failure, muscle metabolism, nutrition.

Introduction

Fatigue and exercise intolerance are the most important symptoms of chronic heart failure. Until recently, these symptoms were considered to be the direct consequence of reduced peripheral perfusion due to degradation of cardiac performance, and were often accompanied by muscle atrophy. In recent years, however, the hypothesis that a defect in the metabolism of skeletal muscle, which is more directly responsible for intolerance than chronic heart failure in patients with the latter condition, has become increasingly evident. Nuclear magnetic resonance studies have demonstrated, both in experimental models of heart failure and in patients affected by chronic heart failure, that there are intrinsic debilitating abnormalities in the skeletal muscles of the arms and legs. Wiener et al. used nuclear magnetic resonance to study muscles of patients with chronic heart failure and showed an increase in the Pi/CP ratio during exercise compared to its normal values at rest; the data were attributed to impaired function of skeletal muscle, but were independent of peripheral flow. In a different muscular district (arm), Massie et al. showed the same features and suggested that exacerbation of anaerobic glycolytic metabolism might be insufficient to maintain adequate ATP content.

These reports convinced investigators that an independent study of skeletal muscle would help explain the processes that contribute to deterioration in chronic heart failure. On the assumption that a direct link exists between skeletal muscle and exercise capacity in chronic heart failure, morphological and biochemical studies focused on skeletal muscle alterations. Mancini et al. found that patients with chronic heart failure frequently...
develop significant skeletal muscle atrophy; they suggested that atrophy contributes moderately to reduced exercise capacity. This was taken to imply that morphological alterations in the muscle may be secondary to an intrinsic modification, causing a basic reduction in performance capacity. By using muscular biopsy, Sullivan et al. demonstrated a reduction in the activity of the most important enzymes involved in aerobic metabolism concomitant with mitochondrial abnormality. This reduction suggested that degradation of the oxidative capacity of skeletal myocytes caused a reduction in muscle efficiency. Thus, it emerges that the morphological modifications of skeletal muscle (such as atrophy) are subordinate to the muscle's internal metabolic processes. Nonetheless, it is evident that a complete study should take into account the metabolic processes that are the cause or effect of the structure and activity of the muscle.

Regardless of what we have learned from the studies above, a proper understanding of skeletal muscle should take into consideration the supply and form of nutrients that support metabolic action in the muscle. If the transformation of the intake of nutrients into energy is obstructed owing to a particular cycle, this will most likely influence the potential action of the skeletal muscle. This underlines the inadequacy of the previous studies on skeletal muscle in chronic heart failure; they failed to consider the food intake of the patients nor did they describe the conditions and the timing of their evaluations. This turns out to be of consequence, because calorie intake and the type of macronutrients ingested influence the hormone profile of patients, thereby causing a change in insulin, cortisol and catecholamine concentrations in the blood. Furthermore, since these hormones are involved in the main metabolic cycles, they can modify the activity of the enzymes. Thus, timing of food intake becomes important, as the ability of the muscle to transform calorie intake into energy for use in external work must be considered in evaluating both metabolic aspects of the peripheral muscle and exercise capacity in patients with chronic heart failure.

Therefore, to gain insight into the energy profile of skeletal muscle, as well as of enzyme activity likely to influence the functioning of muscle, we investigated patients selected for: (1) severe clinical and functional impairment (patients with advanced heart failure have more deranged skeletal muscle metabolism); (2) non-metabolic status; (3) normal calorie and macronutrient intake. Our aim was to better characterize the role of skeletal muscle in chronic heart failure by quantifying the substances that contribute to function in the myocytes. Our results might be of help in determining the contribution of skeletal muscle to the chronic heart failure syndrome.

Methods

Patients

Fifteen males with severe chronic heart failure were studied. Patients belonged to selected New York Heart Association (NYHA) class III (long-term heart failure, that had lasted for more than 18 months) despite individualized medical treatment (ACE inhibitors, diuretics, digoxin, vasodilators). Moreover, all had a left ventricular ejection fraction <30% (incremental treadmill exercise) and a peak VO₂ <14 ml·kg⁻¹·min⁻¹ (by echocardiography test), due to fatigue (Table 1). All patients were in a stable clinical condition.

All patients were hospitalized in the Heart Failure Unit for assessment or re-assessment of indications for cardiac transplant and, in this context, underwent right heart catheterization. None was diabetic, or showed a positive test for oral glucose tolerance.

Nutritional evaluation

All patients underwent anthropometric assessment. The following criteria were used for malnutrition: (1) actual body weight less than 90% of ideal weight; (2) triceps skinfold thickness of the nondominant arm less than the 5th percentile of normal values; (3) a midarm muscle area less than the 5th percentile of normal values. All the patients with normal nutritional status were selected.

Oral intake calculation

In order to verify normal macronutrient and calorie intake, the type and weight of cooked or fresh food was recorded and converted into the raw equivalent when necessary for 3 days before biopsy. Energy intake (kcal·day⁻¹) was 1948 ± 175: 14 ± 2% of energy intake from protein, 25 ± 4% from lipids and 61 ± 5% from carbohydrates.

Skeletal muscle biopsy

Biopsies of the vastus lateralis muscle were taken using Bergstrom's needle technique. The specimens obtained were immediately frozen in liquid nitrogen and

| Table 1 Clinical, haemodynamic and functional characteristics of 15 CHF patients |
|---------------------------------|---------------------|--------------------|
| Patients                        | 15                  |                    |
| Age (years)                     | 52 ± 1              |                    |
| Left ventricular ejection fraction (%) | 21 ± 1-5         | (nv 59:2 ± 1.5)    |
| Peak VO₂                        | 104 ± 0.8           |                    |
| Body weight (kg)                | 71.5 ± 1.9          |                    |
| Actual/ideal body weight (%)    | 103 ± 2             |                    |
| Triceps skinfold (mm)           | 11 ± 0.9            |                    |
| Midarm muscle area (cm²)        | 43 ± 1.4            |                    |
| Cardiac index (l·min⁻¹·m⁻²)     | 1.9 ± 0.08          | (nv 2.5-4.2)       |
| Pulmonary wedge pressure (mmHg) | 23.1 ± 2.8          | (nv 4-5-13)        |
| Right atrial pressure (mmHg)    | 8.7 ± 1.5           | (nv 1.5)           |

nv = normal value.
stored until analysis. All subjects had rested for more than an hour in a post-absorptive state, after a 16 h overnight fast.

The control group for muscle biopsy comprised seven subjects with a mean age of 58·1 ± 1·4 years; all had a negative medical history, physical examination and ECG. These subjects were selected from those who had undergone muscle biopsies in our laboratory. The selection criterion used was age comparable to that of the patients with chronic heart failure. Both patients and normal subjects gave their written consent to enter the study.

Hormones

All samples of venous blood for determination of catecholamines were collected at the same time, after patients had been in a clinostatic position for at least 30 min. Cortisol and insulin levels were measured in the morning, with the subjects fasted.

Plasma catecholamine concentrations were measured using high performance liquid chromatography with electrochemical detection as previously described. Insulin and cortisol blood levels were determined by the radioimmunological method (Cord-CT radioimmuonasay Kit CIS France, Coat A Count Insulin- D.P.C., L.A., California).

Biochemical investigations

For metabolite determination a muscle sample was removed from the liquid nitrogen and immediately powdered (Microdisemembrator Braun) by two passages of 45 s each. The powdered frozen muscle was diluted 1:10 with 0·6 N HClO₄ for acid deproteinization. From the homogenate obtained by two passages in Ultra Turrax of 1 min each, 0·2 ml were submitted to glycogen enzymatic hydrolysis for subsequent spectrophotometric glycogen determination.

The homogenate not submitted to enzymatic hydrolysis was centrifuged at 1500 × g for 15 min in a refrigerated centrifuge (Beckman J2-21; rotor JA-20); the supernatant was neutralized at pH 6 with 2 M KHCO₃ and recentrifuged at 1500 × g for a further 15 min. The extract, kept at a temperature of 0 °C to 4 °C, was immediately used for the spectrophotometric determination of: (a) glycolytic metabolites: glucose, glucose-6-phosphate, pyruvate, lactate; (b) Krebs' cycle intermediates: citrate, α-ketoglutarate, malate; (c) energy store and mediators: AMP, ADP, ATP and creatine phosphate. Pyruvate, lactate, citrate, α-ketoglutarate and malate were determined spectrophotofluorimetrically (Perkin-Elmer LS-5), whereas the other metabolites were determined spectrophotometrically (Beckman 35).

Metabolite concentrations were expressed as μmoles of glycosidic units per gram of fresh tissue for glycogen, and as μmoles·g⁻¹·min⁻¹·mg⁻¹ of protein.

To determine enzyme activities, another sample of muscle was washed with a cold solution of sucrose-EDTA (sodium salt), quickly freed from the muscle sheath and weighed. The tissue was then finely minced, subjected to homogenization by a Polytron tissue processor (Kinematica Instruments) for 5 s and subsequently homogenized in 0·25 M sucrose-1 mm EDTA in a pre-cooled Potter-Braun S homogenizer. The homogenate was diluted with 0·25 M sucrose-EDTA: 1 g of tissue in 10 ml of sucrose solution. This homogenate was then centrifuged at 800 × g for 15 min in a refrigerated centrifuge (Beckman J2-21; rotor JA-20). The supernatant fluid thus obtained was partly used for determining enzyme activities in the crude extract, and/or for protein evaluation and partly stored. The sediment was rehomogenized in 0·25 M sucrose-EDTA and centrifuged at 800 × g for 15 min. The supernatants obtained were centrifuged at 14 000 × g for 20 min. The mitochondrial sediment was gently resuspended in sucrose solution at a final dilution of 100 μg of sediment in 1 ml of sucrose. An aliquot of this preparation was used to assess protein content, while the remaining portion was used to evaluate enzyme activities.

The maximum rate (Vmax) of the following enzyme activities was evaluated in the crude extract and/or mitochondrial fraction: (a) for the anaerobic glycolytic pathway: hexokinase (HK) (EC 2.7.1.1), phosphofructokinase (PFK) (EC 2.7.1.1), pyruvate kinase (PK) (EC 2.7.1.40), lactate dehydrogenase (LDH) (EC 1.1.1.27); (b) for the tricarboxylic acid cycle (TCA): citrate synthase (CS) (EC 4.1.3.7), succinate dehydrogenase (SDH) (EC 1.3.99.1), malate dehydrogenase (MDH) (EC 1.1.1.37); (c) for the electron transfer chain: cytoochrome oxidase (COX) (EC 1.9.3.1); (d) for the NADH/NAD redox couple: total NADH cytochrome c reductase (CCRT) (EC 1.6.99.3); (e) for the aminooacidic pool related to the Krebs' cycle: aspartate aminotransferase (GOT) (EC 2.6.1.1), alanine aminotransferase (GPT) (EC 2.6.1.2).

Enzyme activities were measured by graphic recording for at least 3 min in a double beam recorder spectrophotometer (Beckman 35) and each value was calculated from two blind determinations of the same sample. Enzyme-specific activities were expressed as μmol of substrate transformed × min⁻¹ × mg of protein⁻¹.

Statistical analysis

All biochemical results were expressed as mean ± standard error. Statistical analysis was performed by the one way analysis of variance (ANOVA test) comparing the data obtained from the control group with those obtained from the patients.

Results

The resting hormonal pattern reported in Table 2 shows a slight increase in norepinephrine (315 ± 17 vs
278 ± 75 pg·ml⁻¹) and in cortisol (516 ± 13 vs 308 ± 94 nmol·l⁻¹), in patients in comparison with controls, while insulin levels were normal. The cortisol/insulin ratio was slightly increased (77 ± 4 vs 32 ± 12).

Biochemical analysis of the muscle samples revealed a downward trend in adenosine triphosphate (ATP) in patients with chronic heart failure compared with controls, although these differences had no statistical significance (Fig. 1). Also the value of creatine phosphate (CP) and the CP/ATP ratio (Fig. 1) was significantly lower in this group of patients (5 ± 1 vs 13 ± 1 umoles·g fresh tissue⁻¹). The energy charge potential in patients with chronic heart failure was 0.89 ± 0.02, similar to that reported for normal subjects in the literature. Glycogen content in the muscle was significantly reduced in patients with chronic heart failure in comparison with controls (46 ± 5 vs 77 ± 6 umoles glycosidic units·g fresh tissue⁻¹), whereas the glycolytic metabolites did not differ in the two groups (Fig. 1).

Enzyme activities for the anaerobic pathway in patients with chronic heart failure and controls are shown in Fig. 2. Hexokinase, phosphofructokinase and lactate dehydrogenase did not differ between the two groups, while pyruvate kinase was significantly decreased in the patients with chronic heart failure (1009 ± 79 vs 1625 ± 26 nmoles·min⁻¹·mg protein⁻¹).

The analysis of the enzyme activities related to the tricarboxylic acid cycle (TCA) showed that neither citrate synthase nor succinate dehydrogenase differed between the groups, while both the activities in the amino acidic pool related to the Krebs’s cycle were significantly increased in patients with chronic heart failure (alanine aminotransferase 964 ± 79 vs 425 ± 34, aspartate aminotransferase 515 ± 44 vs 291 ± 56 nmoles·min⁻¹·mg protein⁻¹) (Fig. 2).

As regards the NAD⁺/NADH redox couple, total NADH cytochrome c reductase activity was significantly increased in patients with chronic heart failure.

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<th>Table 2 Resting hormonal pattern in the patients</th>
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<td>Patients</td>
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<td>Norepinephrine (pg·ml⁻¹)</td>
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<td>Cortisol (nmol·l⁻¹)</td>
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<td>Insulin (mcU·ml⁻¹)</td>
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**Figure 1** Concentrations of some metabolites and energy mediators related to muscle energy metabolism in patients affected by chronic heart failure (■) and controls (○). Statistical analysis: analysis of variance (ANOVA test); controls vs patients: *P<0.05. AMP=adenosine monophosphate; ADP=adenosine diphosphate; ATP=adenosine triphosphate; CP=creatine-phosphate; ECP=energy charge potential.
failure compared with controls (128 ± 14 vs 68 ± 6 nmol min⁻¹ mg protein⁻¹) (Fig. 2). However, there were no differences in cytochrome oxidase activity (Fig. 2).

**Discussion**

Our knowledge about the role of the skeletal muscle in chronic heart failure has grown in the recent years. It has been demonstrated, for example, that the peripheral area is impaired by suboptimal perfusion²⁶. It has also been hypothesized that underperfusion is due to a reduction in cardiac output and vasodilatation due to neurohormonal activation, which results in the fatigue caused by intramuscular lactic acidosis¹¹. Patients with chronic heart failure therefore became exhausted with exercise that normal subjects could perform easily.

However, recent significant studies suggested that skeletal muscle in chronic heart failure is characterized by reduced levels of mitochondrial-based enzymes⁹,¹⁹ and muscle atrophy⁸,²⁰. With this view both these phenomena could be directly attributed to deconditioning of the muscle, to malnutrition¹¹,¹⁸, the increased catabolic state as a result of heightened sympathetic stimulation, increases in serum cortisol ACTH and cytokines⁸,²²,²⁴.

We took the results of these recent studies as fundamental guidelines for our investigation of the biochemical aspects of peripheral muscle in patients with chronic heart failure. Our findings confirm the study of Wilson et al.¹¹ and Mancini et al.¹⁸, but tend to shift the interpretation of their results towards a more fundamental role of muscle metabolism as the direct cause of debilitated skeletal function, at least in patients with severe chronic heart failure without nutritional impairment.

Figure 2  Enzyme activities (mean ± SE) evaluated on crude extract and on mitochondrial fraction in patients affected by chronic heart failure (●) and controls (○). Statistical analysis: analysis of variance (ANOVA test); controls vs patients: *P<0.05. HK=hexokinase; PFK=phosphofructokinase; PK=pyruvate kinase; LDH=lactate dehydrogenase; CS=citrate synthase; SDH=succinate dehydrogenase; MDH=malate dehydrogenase; GOT=aspartate aminotransferase; GPT=alanine aminotransferase; CCRT=total NADH cytochrome c reductase; COX=cytochrome oxidase.
Atrophy

The exact mechanism of muscle atrophy in chronic heart failure is not known, but it is very likely caused by chronic physical deconditioning.

Wilson et al. found that in 22 patients affected by chronic heart failure, on which gastrocnemius muscle biopsies were performed, there was a decrease in type II fibres. An increase in hydroxyacyl CoA activity, a mitochondrial based enzyme involved in fatty acid metabolism, was also noted. This suggests that both fibre atrophy as well as changes in enzyme activity may alter skeletal muscle metabolism in patients with chronic heart failure. These findings were confirmed by other authors.

Mancini et al. further equated fibre atrophy to a more generalized condition of skeletal muscle atrophy by performing anthropometric measurements on 62 patients. They noted atrophy of the skeletal muscle in more than 60% of these patients concluding that the less muscle is available for a specific workload, the more performance will be impaired.

Mancini et al. hypothesized that skeletal muscle atrophy was very probably the result of deconditioning, which is inevitably completed by a reduction in mitochondrial-based enzymes. Miyagi et al. demonstrated a relationship between muscle mass of the legs, exercise capacity and daily life activities.

It has been also noted that calorie-protein malnutrition causes atrophy and enzyme changes, and an inadequate nutritional status is frequently observed in patients with chronic heart failure for which the major cause is anorexia. To overcome this problem we selected patients with normonutritional status and normal calorie and macronutrient intake, and performed the muscle biopsy after 16 h of overnight fasting and rest. The aim was to give prominence to muscular energy charge and to the principal metabolic pathway cycles responsible for free cellular energy in known nutritional conditions. In this study we did not evaluate the morphology of the muscle or the fibre type. This was because we had decided to use the entire muscular specimens obtained from the biopsy for the biochemical and enzymatic evaluation.

Energetic charge and enzymatic activities

Wiener et al. and Massie et al. showed that chronic heart failure patients have intrinsic metabolic abnormalities. In fact, using a finger flexion test, they showed that there was a rapid decline in CP and pH indicating greater ATP utilization. Wilson et al. found similar results; they confirmed the CP decline and rise in inorganic phosphate (Pi). In addition, the slope of the relationship between Pi/CP, an index of mitochondrial oxidative metabolism, is steeper in patients with chronic heart failure, suggesting impairment in the peripheral muscle. Assuming that CP could not be resynthesized, and that changes in intracellular pH reflect lactate accumulation, they found that patients had a slightly higher production of lactate and consumption of ATP. These findings also suggest that patients with chronic heart failure exhibit an increased glycolytic metabolism and may be metabolically less efficient in relation to physical exercise.

Our patients displayed different findings. In fact, our data showed that muscular cells of patients with chronic heart failure were almost completely and normally viable from the energy point of view, having a potential energy charge (ECP) of 0.9, which is very near to the ECP of 0.9 which is presently considered the normal value. An ECP of 0.89 indicates that adenine nucleotides in the cell are predominantly present as ATP. However, the association of weak ATP reduction with the net reduction in glycogen and CP suggest a higher rate of consumption of energy than of production. A similar metabolic pattern is present in healthy subjects only during moderate physical activity.

Contrary to Sullivan et al. data on extracted biopsies and on Massie's data on nuclear magnetic resonance, our patients with chronic heart failure showed a 41% reduction in glycogen and a 70% reduction in CP as compared to controls. However these authors did not specify whether they performed the studies in fasting patients or not. It is important to note that glycogen intracellular deposit depends on alimentary carbohydrates and energy intake within the previous 24 h. The food energy intake in our patients was adequate with a carbohydrate contribution equivalent to 60% of total calories. Therefore, to explain the reduction in glycogen, we hypothesize an increase in glycogen breakdown. Physiologically, in resting subjects, this increase is due to adrenergic stimulation and/or to catabolic/anabolic hormone imbalance.

In the patients in the present study we could find no significant increase in catecholamines, probably because of the stable conditions and the optimal tailored treatment. Nevertheless, a catabolic/anabolic hormone imbalance was suspected because of a higher concentration of cortisol and a definitely higher cortisol/insulin ratio with respect to normal values. These data suggest that inhibition of glycogen breakdown carried out by insulin is actively opposed by cortisol. It is important to stress that, in completely resting patients, the reduction in the concentration of glycogen represents, in practice, a limiting factor in both aerobic and anaerobic processes.

We also found a significant reduction in the muscular content of CP in patients with chronic heart failure in contrast to a normal concentration of ATP. A decreased CP/ATP ratio indicates that the balance CP→ATP had shifted towards the right value and that the mitochondrial synthesis of ATP is not available or sufficient to restore CP reserves.

The discrepancy between these results and those of Sullivan et al. and Massie et al. could derive from the different experimental design; in fact, in our study the patients were on prolonged overnight fasting. However, our controls were also similarly fasted overnight.
and did not display a similar reduction in CP/ATP. Therefore, we believe that the reduction in nutrient supply in patients with chronic heart failure 'per se' could not determine the low CP concentration, but it could be induced by a hypercatabolic state. The CP reduction in resting patients indicates an impaired anaerobic pathway, implying a limitation of the available energy which is important at the start of any physical activity.

As regards the three key enzymes in the glycolytic pathway in our patients, hexokinase and phosphofructokinase were unchanged while pyruvate kinase activity was repressed. Normal hexokinase and phosphofructokinase suggest a normal breakdown of glucose in the first step of glycolysis occurred. Pyruvate kinase decrease means that the glycolytic pathway was reduced in the final stage, allowing less pyruvate to enter the oxidative cycle. It follows that there is a tendency towards reduced utilization of glucose in resting and fasting patients, suggesting less efficient ATP production.

In spite of the reduction in glycolytic activity and probably a reduction in the entry of glucose into the aerobic metabolic cycle, the activity of the tricarboxylic acid cycle (TCA) in patients with chronic heart failure was similar to that of the control group. This suggests that there was a higher availability of alternative substrates to glucose, such as acyl-CoA, from fatty acids and amino acids. Moreover, the patients had been fasting for 16 h when physiologically major oxidation of fatty acids and amino acids, at the muscular level, occurred.

Our results revealed that in severe chronic heart failure there is intense activity of the transamination processes, indicating the existence of a lively exchange between aminoacids and intermediates of the TCA cycle. In this process, the pyruvate a-ketoglutarate system is mainly involved. Higher transamination reactions suggest both a major supply of substrates for the TCA cycle and an increase in the formation of alanine. This is the principal aminoacid in gluconeogenesis, a process which took place in our fasting patients. It is possible that the main activity of the transamination processes was partially due to the high cortisol/insulin ratio, since cortisol induces an increase in intracellular transamination.

We could not investigate enzyme activity in relation to beta-oxidation of fatty acids in our patients, and so we have been unable to judge the velocity of breakdown of fats. We hypothesize that the prolonged night fasting increased the utilization of fats, although a reduction in 3-hydroxyacyl CoA and in carnitine may exist in the same patients.

It is important to stress that together with a normal TCA cycle we found greater activity in the tricarboxylic acid cycle (TCA) in patients with chronic heart failure, while cytochrome oxidase activity was repressed. Oxidase, one of the molecules involved in this process, was weakly reduced. At present we are unable to give a biochemical reason for this discrepancy, but we hypothesize that impairment in the final stage of ATP formation would have occurred. Drexler's results confirm our hypothesis; he found a positive reaction for cytochrome oxidase only in 17% of patients with chronic heart failure compared to 62% of control subjects. Thus, oxidative capacity seems to be reduced in patients with chronic heart failure.

Considering the above discussed data, our results are contrasting. On the one hand, we found a higher cellular energy charge and near normal ATP concentration; on the other there was an important reduction in glycogen and CP. Also, as regards the metabolic process, we found a reduction in glycolytic activity in spite of a normal TCA velocity, an increase in transaminations as well as electronic transport, and normal cytochrome oxidase activity.

To explain these contradictions which are only apparent, we believe that an increase in mitochondrial respiration occurred in the patients with chronic heart failure studied by us, but the rate of ATP production was inferior to the rate of utilization. We base our hypothesis on the observation that a value of ECP above 0.89 indicates that the metabolic reaction that consumes ATP exceeds the one that synthesizes it. Consequently, a normal cellular concentration of ATP at rest does not necessarily indicate a normal balance in the relationship between production/utilization of energy, unless both creatine phosphate and aerobic metabolism velocity are normal.

To explain these observations, we postulate that an increase in cellular consumption of ATP occurs due to an increase in cellular work to preserve its integrity and probably to maintain the endocellular homeostasis. Wagner et al. demonstrated that calcium overload occurs in myocytes of the skeletal muscle of animals affected by chronic heart failure. The phenomenon has been correlated with abnormal membrane proteins such as an increase in the number of calcium channels sensitive to voltage.

In conclusion, normonutritive patients with severe heart failure show an imbalance in the ratio of energy production/utilization. The impairment is probably due both to a decrease in production and an increase in consumption for a greater cellular work and/or hypercatabolic state.

The clinical and therapeutic implications of these findings need to be investigated further.

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