Human Skeletal Muscle Energy Metabolism during and after Complete Tourniquet Ischemia

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The extent of cellular metabolic deterioration and its reversibility was studied on human skeletal muscle needle biopsies during operations in bloodless field. The tissue levels of high energy phosphates and glycolytic metabolites were analyzed after various times of tourniquet ischemia and compared to contralateral control extremity levels. In the ischemic extremity the phosphocreatine (CrP) levels decreased by 40% within 30-60 min and after 60-90 min a 60% reduction was found. No significant ATP changes occurred. Lactate levels increased by 225% after 30-60 min and by 300% after 60-90 min. The glucose and G-6-P levels increased slightly and indicated glycogenolysis. The rate of the metabolic changes decreased with ischemia time. In the control leg no significant metabolic changes could be seen. After the release of the tourniquet there was a rapid restoration of the phosphagen content and clearance of lactate in the ischemic leg. Near control levels of these substances were seen already after 5 min. The present results show that clinical tourniquet ischemia of up to 90 min duration produces less pronounced metabolic alterations than those seen in working muscle.

THE USE of tourniquet for obtaining a bloodless field is an essential and accepted tool for extremity operations. The Esmarch bandage[11] initially used was known sometimes to induce various degrees of postoperative nerve palsy. This type of complications is rather rare since the use of pneumatic tourniquet was introduced.[6] It is, however, well known that the risk for complications increases with the time of ischemia and that continuous tourniquet times of less than 2 hours may be considered as relatively safe.[3] The reason for complications, when occurring, has often been discussed either as a consequence of direct pressure of the tourniquet on a nerve[8] or as due to ischemia-induced tissue changes.[7,31] For a proper functional state the peripheral nerves are dependent on an adequate supply of oxygen and a functioning intraneuronal microvascular system. Rather short ischemia times of 25-30 min have been shown to result in sensory recovery within one minute but with a delay of fully restored motor function of 10-17 min.[19,28] The time for reestablishing of intraneuronal circulation has been considered as important for recovery and the use of heparin to prevent microvascular blockage has been reported beneficial in animal experiments.[25] An extensive study by Lundborg[22] has experimentally shown, however, that the intraneuronal microcirculation in rabbits is completely restituted after a circulatory arrest of 6-8 hours ischemia and thus has a wide "margin of safety."

That direct metabolic changes may play an important role is evident since Paulucci[26] and Fogliani[12] found that the release of "constrictors" applied to one or both thighs of experimental animals for more than 3 hours resulted in a severe decrease of blood pressure and death. Rat experiments have shown significant local metabolic and energy changes.[32] Wilson and Roome[35] also considered that absorption of metabolites from the ischemic limb was the main reason for the primary fall in blood pressure.

The cellular metabolic changes during clinical ischemia have often been more indirectly approached by studies based on venous blood parameters in the ischemic extremity.[30,34] The present study was undertaken directly to study the effect of tourniquet ischemia on the cellular metabolism and especially on high energy phosphate level of human skeletal muscle. The adenosintriphosphate (ATP), phosphocreatine (CrP), glucose-6-

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phosphate (G-6-P), glucose and lactate levels of needle biopsies of skeletal muscle were determined preoperatively after various times of ischemia, and after release of the tourniquet and also compared to the levels in the non-ischemic control extremity during the course of operation. Comparisons were made to the effect of routine operations on the corresponding muscle metabolite levels.

Material and Methods

From otherwise metabolically healthy patients (age range 3.5 - 16 years) biopsies were taken from the lower extremity after the induction of anesthesia, at the end of the complete tourniquet ischemia period and in some cases, ischemic for 60-90 min, also 5 and 10 min after the release of the tourniquet. After the same time periods biopsies were taken from the contralateral nonischemic leg. These patients were operated on for knee joint lesions or certain low leg fractures.

Healthy children of similar age range who were to be operated upon for minor defects such as hernia inguinalis, retentio testis etc. were also used for obtaining more extensive control data. Skeletal muscle biopsies were taken immediately after the induction of anesthesia to prevent effects caused by various times of anesthesia. After barbiturate induction, N_2O, O_2, halothane anesthesia was used in all cases.

All the biopsies were taken from the vastus lateralis muscle using the needle biopsy technique described by Bergström.[1] Skeletal muscle samples weighing 20-30 mg wet weight were obtained. No complications or major discomfort due to the sampling were observed. Within a few seconds after the sampling the biopsies were frozen in liquid nitrogen. The frozen biopsies were homogenized at 0 C in 3 M perchloric acid (300 μl) and neutralized with 2 M $KHCO_3$ (850 μl). After centrifugation the supernatants were collected and stored frozen at –20 C for subsequent analysis of the water soluble metabolites. The pellets were used for protein determinations with the Folin phenol reagent technique according to Lowry et al.[21] using crystalline bovine serum albumin as a standard.

To obtain the relation between fresh weight and protein content with the above Lowry technique, some samples were weighed fresh with a recording Cahn RG Electrobalance enabling direct correction for evaporation during the weighing by extrapolation to the wet weight value at the taking of the biopsy. After the weighing the samples were frozen in liquid nitrogen, homogenized as above and the pellets were used for protein determinations while the supernatants were discarded. Such analysis made it possible to calculate the metabolite levels both on basis of protein as well as on basis of fresh weight. Data reported in literature are often based on fresh weight values, but according to our experience, protein determinations give less variation and thus more reproducible tissue metabolite levels. Due to the rapid decomposition, especially of the high energy phosphate compounds, weighing at a temperature of −20 C or even lower is necessary for fresh weight registrations of biopsies subsequently to be analyzed for metabolites. It was not possible for us to obtain satisfactory weighing reproducibility at such a low temperature, and therefore the metabolite data presented below are given per amount of protein. The above experiments, however, make it possible to recalculate the data on fresh weight basis to be able to compare the obtained skeletal muscle levels with such previously reported for human skeletal muscle.

The analysis of the tissue metabolites were performed enzymatically mainly according to Lowry et al.[20] but with minor modifications according to Karlsson.[17] The purified substances and enzymes were obtained from Sigma and Boehringer. The enzymatic reactions were carried out in prematched test tubes (reagent volume 3 ml) in a Beckman ratio fluorometer measuring the NADPH or β-DPNH fluorescence. The ATP, CrP, glucose and G-6-P analyses were carried out in Tris media, pH 7.5, containing 5 mM $MgCl_2$ and BSA 0.01%. For lactate analyses a hydrazine-glucine buffer was used. The fluorescence of each of the skeletal muscle metabolites studied was quantified by analyses of standard solutions of metabolites in identical test media.

Differences in the mean skeletal muscle metabolite levels between the various groups of patients were tested with the t-test.

Results

The mean skeletal muscle levels of ATP, CrP, G-6-P, glucose and lactate for preoperative control patients are given in Table 1. Comparison is made between the levels obtained using protein as reference standard and those calculated on fresh weight basis after determination of the protein-fresh weight relation. The obtained protein content was 9.8%, S.D. ± 0.5% of the wet weight. The tissue levels of ATP, CrP, G-6-P and lactate obtained are in agreement with previously reported values for resting human skeletal muscle.[10,17] No significant difference was

TABLE 1. *The Skeletal Muscle (m. vastus lat.) Content of ATP, CrP, G-6-P, Glucose and Lactate of Pediatric Patients Prior to Operation For Minor Surgical Problems.*

	mmoles x 10^{-6}/mg protein	mmoles x kg^{-1} fresh weight
ATP	44.9 ± 2.4	4.4 ± 0.2
CrP	154.0 ± 10.5	15.1 ± 1.1
G-6-P	5.9 ± 0.9	0.6 ± 0.1
Glucose	15.2 ± 1.9	1.5 ± 0.2
Lactate	16.2 ± 1.7	1.6 ± 0.2

* Comparative values using fresh weight or protein as reference standard. S.E.M. values given. n=16.

TABLE 2. *ATP, CrP, G-6-P, Glucose and Lactate Content in Mmoles x 10^{-6}/mg Protein in Skeletal Muscle Needle-biopsies of Control Patients Preoperatively; Of Patients Undergoing Lower Extremity Operations Using Tourniquet for Complete Ischemia for 30 to 90 min; And of Patients (Ischemic for 60 to 90 min) 5 and 10 min After Release of Tourniquet*

	Preop	30-60 min complete ischemia		60-90 min complete ischemia		5 min after release of tourniquet		10 min after release of tourniquet	
		I	C	I	C	I	C	I	C
	n=16	n=5	n=4	n=3	n=3	n=3	n=3	n=3	n=3
ATP	44.9 ±2.4	56.2 ±8.3	61.3 ±3.5	61.9 ±2.4	53.4 ±4.7	48.6 ±4.1	41.0 ±2.4	52.6 ±1.5	53.6 ±1.1
CrP	154.0 ±10.5	80.6 ±23.3	141.2 ±11.8	62.2 ±15.3	118.5 ±12.7	126.7 ±11.1	130.3 ±3.5	139.7 ±4.6	133.6 ±2.2
G-6-P	5.9 ±0.9	17.7 ±2.9	8.8 ±0.6	17.9 ±2.6	10.6 ±1.5	7.0 ±3.1	5.3 ±0.4	12.1 ±2.3	12.6 ±1.2
Glucose	15.2 ±1.9	17.0 ±1.7	12.1 ±1.1	19.2 ±3.8	16.9 ±3.6	12.7 ±3.4	13.9 ±2.4	12.5 ±2.7	10.3 ±1.8
Lactate	16.2 ±1.7	52.7 ±4.4	22.1 ±7.5	64.5 ±10.6	21.5 ±1.1	17.5 ±6.8	14.9 ±2.5	14.6 ±2.8	14.1 ±1.3

NOTE: I—ischemic leg; C—contralateral control leg. S.E.M.—values given.

observed between the tissue metabolite levels of 5-6-year-old children as compared to 15-16-year-old ones.

In Table 2 the ATP, CrP, G-6-P, glucose and lactate content in mmoles x 10^{-6}/mg protein of skeletal muscle during complete tourniquet ischemia are compared to the corresponding levels in control patients and also in the non-ischemic leg of the patients operated. The effect of 5 and 10 min, respectively, of reestablished circulation on the skeletal muscle metabolism in the leg subjected to tourniquet for 60 to 90 min is also given and compared to the corresponding metabolism in the control leg during the same postoperative period. These temporal events are also graphed in Fig. 1 for ATP and CrP, and in Fig. 2 for G-6-P and lactate to make them more easy to survey.

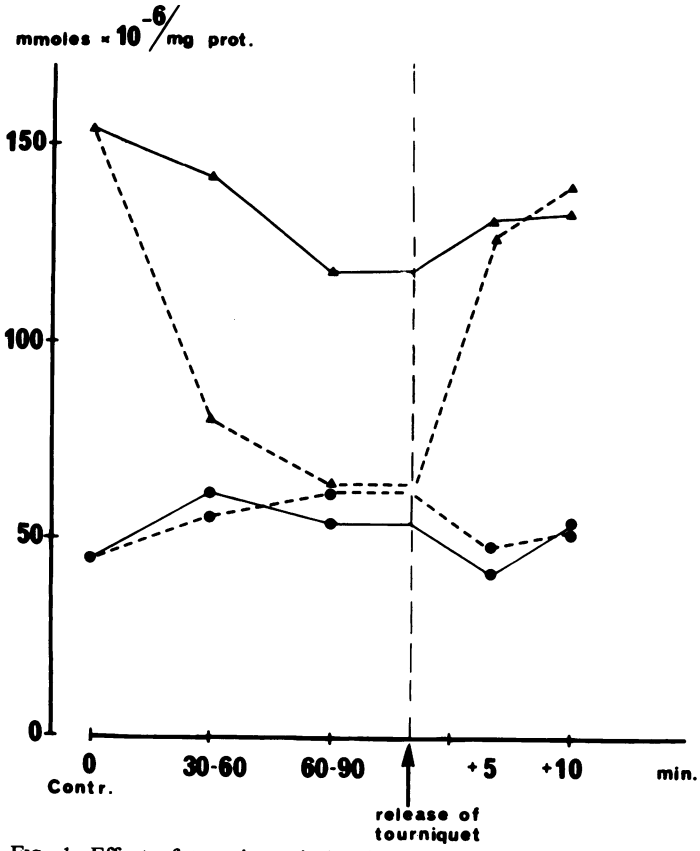

FIG. 1. Effect of tourniquet ischemia on the CrP levels of skeletal muscle from the ischemic leg (--▲-) as compared to that from the contralateral control leg (—▲—) and corresponding ischemic (--●-) and control (—●—) ATP levels. For S.E.M. values consult Table 2.

There were no significant changes in the ATP content of the skeletal muscle during the complete ischemia period as compared to the control patients or to the control leg (Fig. 1). Since the high energy phosphate compounds of skeletal muscle are involved in the following reaction:

Phosphocreatine + ADP ⇆ Creatine + ATP

where CrP is the high energy pool, no such acute changes in ATP can be expected. The reaction, which is catalyzed by the enzyme creatine phosphokinase, is balanced in such a way as to keep essentially all the ADP phosphorylated as ATP at all times, at the expense of CrP. Therefore, changes in the high energy phosphate content of skeletal muscle will mainly be reflected in the CrP level. During the complete ischemia the CrP level reflects such a marked decrease of the total high energy phosphate content (Table 2; Fig. 1). A 30-60 min tourniquet ischemia resulted in a 48% decrease of the CrP level in the ischemic muscle and 60-90 min ischemia in a 60% reduction as compared to the preoperative values. In the contralateral non-ischemic leg also a slight decrease in the CrP level occurred in relation to time but the corresponding decrease was only 8% and 23% respectively. The differences between the non-ischemic control leg and the ischemic leg, however, were significant ($P<0.05$) for both time intervals studied. The release of the tourniquet after 60-90 min of complete ischemia resulted in a rapid increase of the CrP content of the previously ischemic

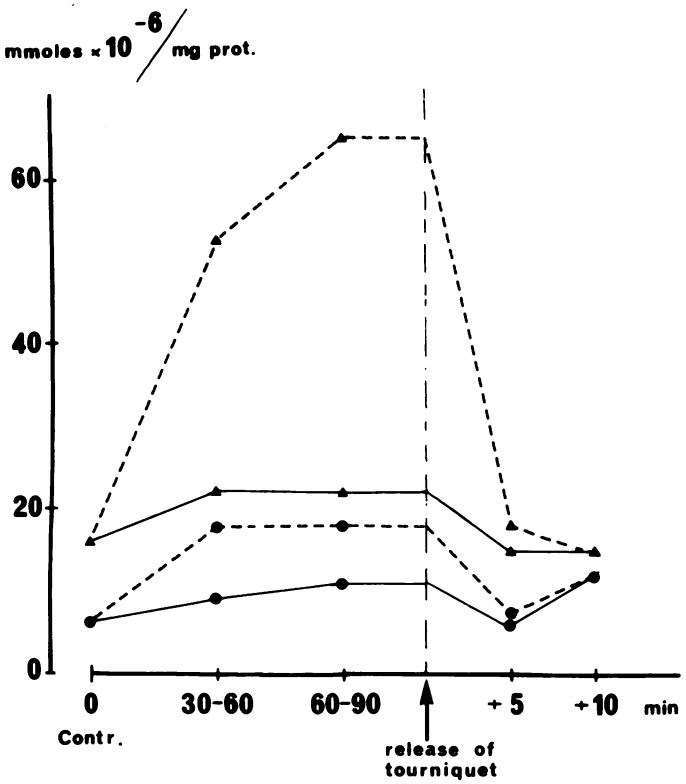

FIG. 2. Effect of tourniquet ischemia on the lactate levels of skeletal muscle from the ischemic leg (--▲-) as compared to that from the contralateral control leg (—▲—) and corresponding ischemic (--●-) and control (—●—) G-6-P levels. For S.E.M. values consult Table 2.

muscle during the first 5 min period. During the following 5-min period a further but slower increase occurred, similar to that observed in the contralateral control leg during the whole postoperative 10-min period studied.

The tourniquet ischemia had a significant ($P<0.05$) initial effect on the muscular G-6-P level, which increased as compared to the contralateral control leg during the first 30-60 min (Table 2; Fig. 2). During the same time period the glucose content also increased ($P<0.05$) in the ischemic leg. After 60-90 min this glucose mobilization in the ischemic leg was no longer so pronounced as compared to the control leg, partly due to an increased mobilization also in the control leg. The release of the tourniquet resulted in a rapid decrease of the G-6-P values during the first 5 min, while during the following 5-min period a slight increase was observed in both legs.

Thirty to sixty minutes of tourniquet ischemia gave rise to a 225% increase of the lactate content in the muscle and prolonged ischemia, in total 60-90 min, to a further but slower increase to totally 300% compared to resting values for control patients. In the contralateral control leg also a slight lactate increase occurred, but the difference to the events in the ischemic leg were significant (30-60 min - $P<0.01$; 60-90 min - $P<0.05$). Postoperatively after the release of the tourniquet a rapid decrease of lactate levels to resting skeletal muscle values were observed during the first 5-min period.

Discussion

The metabolic consequences of complete tourniquet ischemia during human extremity operations have previously been more indirectly studied through blood analysis of parameters such as pH, P_{CO_2}, P_{O_2}, standard bicarbonate, lactate and pyruvate as well as potassium changes during and after ischemia.[30] Previous experimental evidence, however, has clearly shown that in situations, where the normal tissue perfusion is considerably changed, blood parameters are not representative for the actual degree and extent of local cellular changes.[14] Therefore it was considered of importance to study direct cellular metabolic parameters for more exact evaluation of the effect of tourniquet ischemia and also to study the postischemic duration necessary for normalization of such changes.

The present results show a marked decrease in the high energy phosphate pool, a glucose mobilization as evidenced from the increased G-6-P and glucose levels and a lactate accumulation in skeletal muscle during complete tourniquet ischemia. Glycogen changes were not studied as the G-6-P and glucose changes instead were used as indicators of glycogenolytic and glucogenetic activities. Such metabolic changes as the observed ones are of course expected during a relatively longlasting complete circulatory arrest which prevents proper oxygenation and abolishes the nutritive flow to the tissues as well as the clearance of local products from cellular metabolic activities. The ensuing anaerobic metabolism will result in an incomplete glycolysis where the breakdown of one molecule of glucose to lactate results in the production of 2 molecules of ATP as compared to that of about 38 molecules of ATP during complete aerobic oxidation of glucose.

The extent of the observed metabolic changes even after 60-90 min of complete ischemia are surprisingly small in comparison to those observed for example in working human muscle. During maximal exercise in man the CrP decrease and the lactate increase are much more dramatic.[10,17,18] Also a significant decrease of the ATP content is observed during exercise indicating so fast a use of energy that the compensation via the reaction -

Phosphocreatine + ADP ⇆ Creatine + ATP

- is insufficient to keep all the ADP phosphorylated as ATP at the expense of CrP. During exercise the blood flow to the working muscles is also markedly increased, enabling a continuous clearance of metabolites as reflected in changed central blood levels of metabolites.

In the case of tourniquet ischemia a quite different situation is at hand. The skeletal muscle is in a resting condition and only an energy demand sufficient to cope

with the basal metabolic needs is necessary. Due to the low energy expenditure the development of a metabolic deterioration induced by the anoxic situation is slow in spite of the complete circulatory arrest. This is also reflected in the fact that no significant decrease in ATP levels was observed as the time necessary for keeping all the ADP phosphorylated as ATP at the expence of CrP was always sufficient. In the course of the anoxic time period one would expect, however, a linearily increasing or even accelerated metabolic deterioration. No such effect was observed. On the contrary the rate of the metabolite accumulation and the energy phosphate reduction decreased with increasing tourniquet time. This decrease in the rate in relation to time is due to the continuous decrease in temperature in the non-perfused extremity. The metabolic rate of the ischemic skeletal muscle consequently decreases and partly protects the tissues against the harmful effects on anoxia.[2] Animal experiments have also shown that the use of additional local hypothermia during prolonged tourniquet occlusion is protective against destructive changes[24] and similarly local cellular damage during hemorrhagic shock has been shown to diminish during hypothermia.[13,15] It is therefore obvious that the observed metabolic effects of tourniquet operations could be further reduced by the use of additional hypothermia beyond the decrease in temperature obtained due to the circulatory arrest only.

The metabolic changes in the control leg were minor during the same time period. The difference between the changes in the control leg as compared to those reported for patients subjected to routine operations indicates that the circulatory contact with the surgical area has some importance.[9] During general anesthesia without superimposed surgical stress it has previously been shown that no evidence of stress reactions in the organism seems to occur as judged from catecholamine and growth hormone levels or changes in glucose or fat metabolism.[33] In the case of superimposed surgery blood analysis reveals a marked hyperglycemic response which is largest during intra-abdominal surgery.[5] Such effects have been considered evoked by painful stimuli and possibly mediated via release of cortisol. As the routine operations have been shown to produce a somewhat more pronounced metabolic effect in skeletal muscle than that observed in the control leg during tourniquet ischemia[9] the release of cellular products from the operation area could partly be of importance for such an effect. In connection with trauma or shock the release of intracellular products, such as proteolytic enzymes which have vasoactive effects, has often been considered of importance for inducing irreversibility reactions.[23,27] During tourniquet ischemia it is obvious that there is a local accumulation of metabolites which will change the "milieu interieur" for the cells and could affect cellular membrane functions and cause liberation of intracellular substances. The reduction in temperature will further affect membrane characteristics such as the efficiency of the sodium pump.[4] The well known fact that the release of a "constrictor" applied to an extremity for a time period long enough will induce profound hypotension and death,[12,26,35] further indicates that such factors are of importance in connection with complete local ischemia. The studied duration of up to 1.5 hours of complete tourniquet ischemia, however, does not seem to induce any, at least significant, such general effects due to metabolite absorption or liberation of intracellular substances as the release of the tourniquet does not negatively affect the metabolism in the control leg.

The present results also show that after the release of the tourniquet a rapid resynthesis of high energy phosphate and clearance of tissue metabolites occurs. The rapidity with which this normalization occurs is probably enhanced by the postischemic reactive hyperemia which is induced by local factors in the previously ischemic leg. The exact nature of such factors evoking a reactive hyperemia is still obscure, although factors such as reduction of P_{O_2}, or pH, accumulation of C_{O_2} or lactate, ATP, potassium, bradykinin or inorganic phosphate have been discussed as possible mediators.[16] When there is a need for prolonged tourniquet ischemia a release period of the tourniquet for about 10 min after 1.5 hrs of ischemia has been recommended.[3] The present results indicate that such a "breathing spell" is quite sufficient for a relative normalization of the skeletal muscle metabolism and clearance of tissue metabolites.

On the basis of the present investigation it may be concluded that the metabolic changes caused by tourniquet ischemia of up to 1.5 hrs duration are readily reversible. Animal experiments[7] as well as human studies[29] have indicated an occurrence of degenerative-necrotic changes in muscle fibers following tourniquet ischemia. Since our present human data do not show any remaining disturbances of the phosphagens of glycolytic metabolites, the extent of such acute degenerative-necrotic changes must be too small to affect the studied metabolic parameters. Although ischemia experimentally has been reported to cause only minor changes in the microcirculation of nervetrunks even after 6 - 8 hours and result in good junctional recovering of nerve and muscle tissue[22] there seems to be delayed effects appearing days after the temporary ischemia. Histochemical changes in skeletal muscle fibers and motor nerve terminal changes are becoming demonstrable as long as up to 10 - 15 days later.[7] The postischemic period includes a period of tissue edema with additional possibilities for interference with the microcirculation and nerve function. Therefore, the present acute metabolic data on skeletal muscle can not be used as a direct evidence for a wide "margin of safe-

ty'' since possible delayed postischemic reactions were not determined. There is consequently a need for additional clinical studies of tissue metabolism parameters during a more prolonged postischemic time period.

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