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## Glucose Metabolism in Mouse Tumor and Liver With and Without Hyperthermia†

Christian Streffer,\* Sieglinde Hengstebeck,\* and Peter Tamulevicius\*

*We measured levels of glycolytic metabolites in mouse tumor and liver after administering a glucose load of 6 mg/g of body weight and after hyperthermia for one hour at 43°C. Metabolites included glucose, glucose-6-phosphate, fructose-1.6-diphosphate, dihydroxyacetone-phosphate, glycerol-3-phosphate, pyruvate, and lactate, as well as acetoacetate and  $\beta$ -hydroxybutyrate.*

*The combined treatment led to an increase of the lactate level and apparently enhanced glucose degradation. The*

*redoxequilibria states were shifted to the reduced metabolites. It is possible that hypoxia was induced or enhanced, which could have significance for tumor therapy.*

*At later periods after hyperthermia, metabolic alterations occurred that have also been observed in severe diabetes. These alterations occurred in the liver as well. In both situations, such alterations must be considered in connection with potential damage to normal tissue from hyperthermia.*

Metabolic pathways are very well regulated. If temperature changes alter the rate of enzymatic reactions, disorders of the whole pathway can occur. At very high temperatures, denaturation of proteins takes place (1). Damage to cancer cell metabolism by hyperthermia has been reported (2,3). Von Ardenne has suggested that the heat sensitivity of cancer cells can be increased by glucose (4,5). It is assumed that lactate accumulates under these conditions and lowers intracellular pH. In addition, other authors have shown that lowering of the pH value causes a remarkable enhancement of cell killing by hyperthermia (6).

Because glucose metabolism is coupled in several complex ways with the described parameters, it seemed of interest to study its alteration by hyperthermia in an experimental tumor. Tumor therapy is very strongly linked to the tolerance of normal tissues. As glucose metabolism is especially active in liver, hepatic tissue is included in these studies.

An adenocarcinoma was transplanted into inbred mice (C<sub>57</sub> black) by injecting a tumor cell suspension into the muscles of the hind leg. Six days after transplantation, hyperthermia studies were carried out. Glucose (6 mg/g body weight) was injected intraperitoneally directly before the hyperthermia treatment began.

After the mice had been anesthetized, the tumor was heated in a water bath for one hour at 43°C. Temperatures were measured in the tumor and in the neck. In the tumor, the temperature increased from 35.2°C to 42.0°C within 10 minutes. After 30 minutes of heating, it reached 42.7°C and stayed at this temperature until the end of heating. After the treatment was completed, the temperature dropped to less than 30°C and remained at this low level for several hours. In the neck, the temperature also increased to 40.4°C within 30 minutes of heating at 43°C. After treatment, the rectal temperature fell to about 32°C for several hours.

For the metabolic determinations, both the liver tissue and the tumor were removed under anesthesia, homogenized with 4% perchloric acid, and centrifuged. The supernatant was neutralized and used for the determination of glucose, glucose-6-phosphate (G-6-P), fructose-1.6-diphosphate (FDP), dihydroxy-acetonephosphate (DAP), glycerol-3-phosphate (G-3-P), pyruvate, lactate, acetoacetate (AcAc), and  $\beta$ -hydroxybutyrate ( $\beta$ -HO-Bu). The metabolites were measured by enzymatic assays coupled to NAD<sup>+</sup> or NADH (7).

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## Results and Discussion

After the intraperitoneal injection of glucose in a rather high dose (Table I), the glucose level in the liver tissue increased very rapidly and reached its highest level within 10 minutes. After 60 minutes, the glucose level had decreased again. In the hepatic tissue, most of the measured glycolytic metabolites were not considerably altered; only pyruvate and lactate, the end products of glycolysis, were enhanced.

When the hyperthermia treatment was started just after the glucose injection, the glucose content in the liver was the same as without hyperthermia. However, the levels of glycerol-3-phosphate, pyruvate, lactate, and  $\beta$ -hydroxybutyrate had increased. As mentioned earlier, the local heating induced a rise of the temperature in the whole mouse to about 40°C (Table I). These data demonstrate the difficulties in heating tissues locally, and, as a consequence, in limiting the effects of hyperthermia to the local tumor.

The problem of heat dissipation is especially acute with smaller animals such as the mouse and will be less serious in clinical tumor therapy, although it does exist.

In the tumor tissue, glucose increased at about the same rate as in the liver (Table II). The highest levels were obtained 20-40 minutes after the injection (about 14  $\mu$  moles/g tissue). The increases in lactate and in FDP and DAP levels were comparatively small.

With hyperthermia, the glucose and G-6-P in the tumor were reduced, while G-3-P and  $\beta$ -hydroxybutyrate were

increased. Similar changes in G-3-P and  $\beta$ -hydroxybutyrate occurred when a glucose load was given just before the hyperthermia treatment was started. However, under these conditions (glucose plus hyperthermia), the lactate content was also enhanced in the tumor directly after the hyperthermia treatment had ended.

Dickson and Calderwood (8) observed that glycolysis in Yoshida sarcoma of rats was inhibited after hyperthermia treatment in vivo when the tumor tissue was incubated in vitro. Our data appear not to support such a mechanism for the period during hyperthermia, because our finding that the glucose level was lowered by hyperthermia demonstrates a more rapid glucose turnover. In other experiments with mice, we found that the output of  $^{14}\text{CO}_2$  increased during whole body hyperthermia (one hour at 40°C or 41°C) after radioactive-labelled glucose was injected (unpublished results). We have also observed that during whole body hyperthermia a considerable breakdown of hepatic glycogen occurred, and no accumulation of glycolytic metabolites was found (9). However, some hours after hyperthermia the metabolic degradation of glucose was apparently decreased, an effect which agrees with the data of Dickson and Calderwood (8) and which will be discussed later. It was further surprising that most of the glycolytic metabolites besides lactate increased very little.

When we studied rats with a transplantable glioblastoma after chronic glucose infusion, we found a higher rise of G-6-P, FDP and DAP, which coincided with a decrease of the pH value (unpublished results). These data suggest that if hyperthermia inhibits glucose degradation, lactate consumption may be somewhat reduced.

TABLE I  
Content of Metabolites\* in Mouse Liver After Glucose Injection and Hyperthermia

	Control	Glucose** 10 min	Glucose** 60 min	Glucose** and hyperthermia 60 min
Glucose	10.2 $\pm$ 0.2	37.0 $\pm$ 3.0	23.9 $\pm$ 2.0	23.4 $\pm$ 0.1
G-6-P	0.36 $\pm$ 0.01	0.42 $\pm$ 0.02	0.36 $\pm$ 0.02	0.32 $\pm$ 0.02
FDP	0.038 $\pm$ 0.003	0.031 $\pm$ 0.002	0.041 $\pm$ 0.02	0.034 $\pm$ 0.002
DAP	0.035 $\pm$ 0.001	0.035 $\pm$ 0.002	0.043 $\pm$ 0.02	0.038 $\pm$ 0.002
G-3-P	0.52 $\pm$ 0.02	0.51 $\pm$ 0.02	0.70 $\pm$ 0.05	1.35 $\pm$ 0.06
Pyruvate	0.047 $\pm$ 0.003	0.119 $\pm$ 0.011	0.10 $\pm$ 0.01	0.13 $\pm$ 0.01
Lactate	1.95 $\pm$ 0.09	3.29 $\pm$ 0.15	3.67 $\pm$ 0.13	6.10 $\pm$ 0.17
AcAc	0.049 $\pm$ 0.004	0.053 $\pm$ 0.007	0.061 $\pm$ 0.005	0.060 $\pm$ 0.005
$\beta$ -HO-Bu	0.19 $\pm$ 0.01	0.16 $\pm$ 0.01	0.17 $\pm$ 0.01	0.32 $\pm$ 0.02
G-3-P/DAP	14.8	14.6	16.3	35.5
Lact/Pyruvate	41.5	27.5	36.7	47.0
$\beta$ -HO-Bu/AcAc	3.9	3.0	2.8	5.3

\*  $\mu$  moles/g tissue

\*\* 6 mg/g body weight

## Glucose Metabolism and Hyperthermia

It should also be mentioned that hyperthermia plus glucose shifted the redoxequilibria state in the direction of the reduced metabolites (Tables I and II) for the ratios of both lactate/pyruvate and  $\beta$ -hydroxybutyrate/acetoacetate, especially in the tumor. This shift could mean that hypoxia was induced or enhanced under these conditions and could have some relevance to the pH value through the following equation:

$$K = \frac{[\text{lactate}] \times [\text{NAD}^+]}{[\text{pyruvate}] \times [\text{NADH}] [\text{H}^+]}$$

Analogous equations are valid for the other redoxequilibria states. Such changes would have a great significance for tumor therapy and must be investigated further.

In further experiments, the metabolites were measured over a 12-hour period after hyperthermia treatment without glucose injection (Table III). Glucose content decreased sharply, followed by the other glycolytic metabolites including lactate and pyruvate. During this period, the CO<sub>2</sub> output from glucose was also diminished (unpublished results). However, two other strong acidic metabolites,

**TABLE II**  
**Content of Metabolites\* in Transplanted Adenocarcinomas After**  
**Glucose Injection and Hyperthermia**

	Control	Hyperthermia alone	Glucose alone 60 min	Glucose** + hyperthermia 60 min after injection = directly after hyperthermia
Glucose	3.12 ±0.10	1.19 ±0.14	10.96 ±1.19	7.99 ±0.47
G-6-P	1.02 ±0.03	0.65 ±0.04	0.70 ±0.03	1.10 ±0.10
FDP	0.030 ±0.001	0.027 ±0.003	0.048 ±0.002	0.061 ±0.002
DAP	0.036 ±0.001	0.030 ±0.002	0.046 ±0.002	0.054 ±0.002
G-3-P	0.37 ±0.01	0.50 ±0.02	0.36 ±0.01	0.58 ±0.01
Pyruvate	0.22 ±0.01	0.22 ±0.01	0.20 ±0.01	0.17 ±0.01
Lactate	14.3 ±0.3	13.1 ±0.5	16.5 ±0.5	26.5 ±0.6
AcAc	0.017 ±0.002	0.016 ±0.003	0.018 ±0.002	0.014 ±0.002
$\beta$ -HO-Bu	0.097 ±0.007	0.159 ±0.008	0.069 ±0.004	0.15 ±0.01
G-3-P/DAP	10.2	16.7	7.8	10.4
Lact/Pyruvate	65	60	82	156
$\beta$ -HO-Bu/AcAc	5.7	10.0	3.8	10.7

\*  $\mu$  moles/g tissue

\*\* 6 mg/g body weight

**TABLE III**  
**Content of Metabolites\* in Transplanted Adenocarcinomas After Hyperthermia**

	Control	1 hour	6 hours	12 hours
Glucose	3.12 ±0.10	1.59 ±0.12	1.35 ±0.08	1.04 ±0.064
G-6-P	1.02 ±0.03	0.70 ±0.04	0.90 ±0.046	0.44 ±0.036
FDP	0.030 ±0.001	0.022 ±0.002	0.027 ±0.001	0.017 ±0.001
DAP	0.036 ±0.001	0.029 ±0.002	0.029 ±0.002	0.023 ±0.002
G-3-P	0.37 ±0.01	0.31 ±0.01	0.37 ±0.02	0.28 ±0.012
Pyruvate	0.22 ±0.01	0.24 ±0.01	0.17 ±0.01	0.11 ±0.01
Lactate	14.3 ±0.3	11.9 ±0.4	12.1 ±0.4	7.90 ±0.30
AcAc	0.017 ±0.002	0.041 ±0.007	0.058 ±0.009	0.19 ±0.02
$\beta$ -HO-Bu	0.097 ±0.007	0.249 ±0.027	0.338 ±0.030	0.75 ±0.07
G-3-P/DAP	14.8	10.7	12.8	12.1
Lact/Pyr	41.5	49.6	36.7	46.7
$\beta$ -HO-Bu/AcAc	3.9	6.1	5.9	4.9

\*  $\mu$  moles/g tissue

acetoacetate and  $\beta$ -hydroxybutyrate, were greatly increased after hyperthermia. Similar observations were made in the liver tissue of mice after whole body hyperthermia (9). Thus, metabolic alterations occurred which were also found in severe diabetes. These might also contribute to the liver damage which has been observed after clinical hyperthermia applications. The acidic metabolites could be formed from glucose via lactate and acetyl-CoA or from fatty acids.

Thus, glucose metabolism might be important for two reasons. First, the sensitivity of the tumor tissue to hyperthermia might be altered so that blood flow and the oxygen supply can be influenced concomitantly. Second, hyperthermia may induce changes in glucose metabolism over a longer period which could contribute to pathophysiological damage in normal tissues.

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