Effects of Starvation on Glycogen Contents of Heart, Skeletal Muscle and Liver in Several Mammals

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Abstract The effects of short-term starvation on cardiac, hepatic and skeletal muscle glycogen contents were observed using kids, piglets, rats, hamsters and guinea-pigs. 1) Cardiac glycogen; In the kids given a milk-replacer once daily, it was highest at 2 hr after suckling. In the rats given a diet during the beginning of the light period, food consumption decreased it significantly ($P < 0.05$). In rats, hamsters, guinea-pigs and piglets fed *ad libitum*, starvation increased it. 2) Skeletal muscle glycogen; In kids and rats adapted to the restricted feeding, food consumption increased it and starvation decreased it. In the animals fed *ad libitum*, 33 to 59% of glycogen were lost during starvation. The piglets of 2.5 weeks old deposited 21.4 mg/g of glycogen in the feeding period. After 24 hr starvation, 14.3 mg/g of glycogen was stored. 3) Hepatic glycogen; In the animals with *ad libitum* feeding, it fell by over 90% during starvation. In kids and rats with the restricted feeding, food intake increased it. 4) Plasma free fatty acids (FFA); Starvation increased it and food ingestion decreased it significantly ($P < 0.05$). A positive correlation between cardiac glycogen and plasma FFA was not found in the kids. 5) These results show that there is a cyclic change in the skeletal muscle and liver glycogen content, which is characterized as having a peak just after the meal and a nadir just before the meal, and that the cyclic change in cardiac glycogen seems to be affected by an alteration of feeding time.

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Glycogen contents in the liver and skeletal muscle decrease during starvation, whereas the increase in glycogen content during prolonged starvation has been observed in cardiac muscle¹⁻³⁾. These changes have been discussed in connection with plasma free fatty acids (FFA) concentration⁴⁻⁷⁾. It was shown in the previous paper⁸⁾ that in the rat being adapted to the restricted feeding schedule the diurnal change in cardiac glycogen content was similar to that in the serum FFA concentration.

Though it has been supposed that food intake is effective in synchronizing the diurnal change of glycogen content in the liver and skeletal muscle⁹⁾, it has not been clearly shown that the change in the FFA caused by food intake regulates that of cardiac glycogen directly.

In this paper, experiments were done to confirm whether the reciprocal correlation between cardiac and hepatic or muscular glycogen during starvation and the positive correlation between cardiac glycogen and the blood FFA exist on any feeding condition or any animal species.

Materials and Methods

Animals and experimental procedures

Kids; Nine kids were removed from their dams on the second day after birth and maintained in individual metal cages and taught to drink liquid diet from nipple bottle once daily at 0900. Daily amounts of milk-replacer solid were 60 and 82 g for the first 3 weeks and the following experimental period, respectively. The milk-replacer was reconstituted with warm water at a ratio of 1 to 4. After 6 weeks of the feeding, they were divided into three groups of 3 kids of each and killed by the incision of main vessels of the neck after anesthetizing with ethyl ether at 2, 24 and 48 hr after suckling.

Rats with restricted feeding: Male rats of Wistar strain, weighing 160 to 170 g, were caged individually in the room where temperature and humidity were controlled at $22\pm1^{\circ}\text{C}$ and $55\pm5\%$, respectively, with a light period from 0930 to 2130. They were given a standard diet from 0930 to 1130 for 6 weeks until the day before the slaughter. On the day of the slaughter, they were divided into 2 groups of 5 rats. One group was killed just before feeding, *i.e.* 0930, the other group was killed at 4 hr after the start of the meal, *i.e.* 1330. Water was available at all times.

Piglets: Experiment was carried out from April to May. Sixteen male crossbred pigs (Landrace $\times$ Hampshire) of 2.5 weeks of age, weighing about 3 kg, were removed from their dams. Immediately they were divided into two groups of identical body weight. Eight animals of fed group were killed by the incision of main vessels of the neck immediately after dividing, at 0900. Remaining 8 piglets of starved group were placed in metal cage and no food or water was given. They were killed after 24 hr starvation.

Rats, hamsters and guinea-pigs with *ad libitum* feeding: Eight male Wistar strain rats of 4 weeks, seven male golden hamsters of 4 weeks and six male guinea-pigs of 11 weeks of age were caged individually with exposure to natural day and night cycles during the months of May to June. The animals had free access to food and water. After 3 weeks of the feeding, they were divided into two groups of 3 to 5 animals at random at 1100. Animals of fed group were killed by decapitation immediately after dividing and the other animals were killed after 48 hr starvation.

Diets

Rats, hamsters and guinea-pigs with *ad libitum* feeding were given commercial chow pellets (Oriental Yeast Co., Japan). The diet given to rats with restricted feeding was consisted of 20% casein, 5.0% soy bean oil, 70% corn starch and 5.0% other ingredients⁸⁾. Preruminant kids were given milk-replacer solid consisting of 87.2% dried skim milk, 10% lard, 2% lecithin, 0.8% other ingredients⁹⁾. Piglets were given mother's milk freely until the day of experiment.

Analytical methods

In all animals used, samples of heart, liver and skeletal muscle (*M. gastrocnemius*)

were removed from the carcass just after slaughter and they were placed in test tubes containing 30% KOH for the subsequent glycogen analysis. Glycogen contents were determined as previously described⁹⁾. At the time of slaughter, blood samples of kids, piglets and rats with restricted feeding were collected with test tube containing heparin as anticoagulant for the determination of blood plasma FFA concentrations. FFA was determined with the commercial kit (NEFA-Test Wako, Wako Pure Chemical Industries, Ltd, Osaka, Japan).

Results and Discussion

Changes in glycogen contents observed in these experiments were classified into two parts. One was the changes caused by prolonged starvation. The glycogen contents of 48 hr-starved kids, rats, hamsters and guinea-pigs, and 24 hr-starved piglets were considered. The other was 24 hr-cycle changes according to 24-hr feeding schedule. Preruminant kids of 2 and 24 hr starvation and rats with restricted feeding were included. Table 1 summarizes the results of experiments.

Change in glycogen contents during starvation

Aspects of fluctuation in the tissue glycogen were similar to the results shown by others^{1,2,10)}, that is, hepatic and muscular glycogen contents decreased and cardiac

Table 1. Effects of starvation on tissue glycogen contents and plasma FFA concentration.

Treatments	No.	Heart	Muscle	Liver	FFA
		(mg/g wet weight)			mEq/l
Preruminant kids					
2 hr after suckling	3	8.72±1.09 ¹⁾	5.25±0.71 ^a	30.0 ±2.9 ^a	0.298±0.064 ^a
Starved, 24 hr (just before feeding)	3	5.68±0.72	3.44±0.79 ^a	5.43±3.88 ^b	0.779±0.161 ^{ab}
Starved, 48 hr	3	6.13±0.52	1.46±0.11 ^b	1.54±0.15 ^b	1.285±0.035 ^b
Rats with restricted feeding					
Starved, 2 hr ³⁾	5	2.84±0.25 ^a	6.30±0.16 ^a	49.5 ±2.0 ^a	0.468±0.036 ^a
Starved, 22 hr ⁴⁾	5	3.94±0.10 ^b	3.46±0.13 ^b	21.5 ±1.8 ^b	1.200±0.055 ^b
Piglets					
Suckling, <i>ad libitum</i>	8	5.42±0.83	21.4 ±2.8	48.8 ±3.8 ^a	0.188±0.024 ^a
Starved, 24 hr	8	6.51±0.54	14.3 ±1.9	2.60±0.56 ^b	0.537±0.047 ^b
Rats fed <i>ad libitum</i>					
Fed, <i>ad libitum</i>	3	1.24±0.13 ^a	1.79±0.61	44.5 ±9.9 ^a	
Starved, 48 hr	5	4.91±0.21 ^b	0.75±0.20	3.33±0.53 ^b	
Hamsters					
Fed, <i>ad libitum</i>	3	0.92±0.14 ^a	3.94±0.40 ^a	67.2 ±2.5 ^a	
Starved, 48 hr	4	3.13±0.31 ^b	1.63±0.33 ^b	2.49±0.17 ^b	
Guinea-pigs					
Fed, <i>ad libitum</i>	3	3.17±0.18	4.40±0.17	53.0 ±2.6 ^a	
Starved, 48 hr	3	4.60±0.50	2.90±0.56	1.55±0.23 ^b	

1) Mean±standard error of mean. 2) Differences between figures with different superscript are significant at 5% level. 3) Corresponds to 2 hr after the end of feeding time. 4) Corresponds to 22 hr after the end of feeding time (just before feeding).

glycogen increased during starvation. Though cardiac glycogen of the kids was the highest at 2 hr after suckling, it was slightly higher after 48 hr starvation than after 24 hr starvation. It seemed to indicate that prolonged starvation increased cardiac glycogen in the kids.

The changes in glycogen contents in piglets were similar to those in rodents, but muscular glycogen content in suckling group was 4 to 10 times that was observed in other animals. In addition, 14.3 mg of glycogen per 1 g tissue was stored in the skeletal muscle after 24 hr starvation in spite of a significant decrease in hepatic glycogen. It is well known in neonatal mammals that the glycogen contents of liver, heart and skeletal muscle are very high and that immediately after birth they decrease rapidly in the liver and somewhat more slowly in skeletal muscle^{11,12}. It had shown that newborn piglets deposit over 70 mg/g of glycogen in the skeletal muscle¹³. The result obtained in this work showed that muscular glycogen content of neonatal piglets remained at a high level even after 2.5 weeks after the birth.

SUGDEN *et al.*¹⁴ suggested that glycogen of skeletal muscle is an important factor in carbohydrate metabolism during prolonged starvation and a potentially valuable source of blood glucose *via* the Cori cycle. It was shown in this experiment that hepatic glycogen of animals with *ad libitum* feeding fell by over 90% during starvation whereas the loss of muscular glycogen was 33 to 59%. The result supports the view of SUGDEN *et al.*.

Cyclic change in cardiac glycogen contents

In the experiments of 24 hr-cycle changes cardiac glycogen was the highest in the kids at 2 hr after suckling, but ingestion of food decreased cardiac glycogen content in the rats being adapted to the restricted-feeding. The result observed in the rats was the same as those reported previously⁹. These results suggested that there was a cyclic change in the cardiac glycogen content and that the change in the kids was characterized as having a peak just after the meal and a nadir just before the meal and *vice versa* in the rats with restricted feeding.

Most experiments concerning the changes of tissue glycogen so far mainly used animals being fed *ad libitum*¹⁵⁻¹⁷. On *ad libitum* feeding cardiac glycogen as well as muscular and hepatic glycogen were deposited during the time of maximal food intake¹⁶⁻¹⁸. As the rat is a nocturnal animal, it consumes most of its food at night^{16,17}. So the deposition of cardiac glycogen occurs at night. As the kid is a diurnal animal, it consumes milk from its dam during light period. In this experiment, the kid was fed in a light period, *i.e.*, once daily at 0900, so the increase of cardiac glycogen after suckling in the kid corresponds to the deposition of cardiac glycogen at night in the rat of *ad libitum* feeding. But the rat of restricted-feeding of this experiment was given the diet during the beginning of light period. This suggested that an alteration of feeding pattern could affect the cardiac glycogen metabolism.

Starvation increased the plasma FFA concentrations and food ingestion decreased them significantly ($P < 0.05$). This observation was the same as those reported by

others¹⁹⁻²¹). In previous work, as a positive correlation between cardiac glycogen and plasma FFA was obtained in rats of restricted feeding similar to this experiment, we assumed that fluctuation in cardiac glycogen content was regulated by FFA. But a reciprocal relationship between the cardiac glycogen and FFA was reported by GARTHWAITE *et al.* in the rats of *ad libitum* feeding¹⁷). In addition, a positive correlation between cardiac glycogen and plasma FFA was not found in the pruruminant kids of this experiment. Judging from these results it may be concluded that the relationship observed in the previous work³) is an apparent correlation, though the increase in cardiac glycogen content during starvation has been explained by the elevated concentration of plasma FFA⁴⁻⁷). Plasma FFA may play an important role in storage of cardiac glycogen during prolonged starvation but not for diurnal change.

Though glycogen content in the liver and skeletal muscle is primarily affected by feeding and affected little by lighting regimen, it is suggested that glycogen content in the heart is affected by lighting regimen. So when we discuss the mechanism by which cardiac glycogen is controlled, it is necessary to consider the difference in experimental design, especially the relationship between the lighting regimen and the feeding time.

References

- 1) EVANS, G., *J. Physiol. (London)*, **82**: 468-480. 1934.
- 2) ADROUNY, G. A. and J. A. RUSSELL, *Endocrinology*, **59**: 241-251. 1956.
- 3) MATSUMOTO, M. and R. FUNABIKI, *J. Nutr. Sci. Vitaminol.*, **23**: 471-474. 1977.
- 4) NEWSHOLME, E. A., P. J. RANDLE and K. L. MANCHESTER, *Nature*, **193**: 270-271. 1962.
- 5) GARLAND, P. B., P. J. RANDLE and E. A. NEWSHOLME, *Nature*, **200**: 169-170. 1963.
- 6) ADROUNY, G. A., *Am. J. Physiol.*, **217**: 686-693. 1969.
- 7) NEELY, J. R., C. F. WHITFIELD and H. E. MORGAN, *Am. J. Physiol.*, **219**: 1083-1088. 1970.
- 8) FUNABIKI, R., S. IDE, N. EBE, O. TAGUCHI and K. YASUDA, *Agric. Biol. Chem.*, **39**: 791-793. 1975.
- 9) MATSUMOTO, M. and T. HAMADA, *Bull. Nat. Inst. Anim. Ind.*, **34**: 29-35. 1978.
- 10) ÅGREN, G., O. WILANDER, and E. JORPES, *Biochem. J.*, **25**: 777-785. 1931.
- 11) SHELLY, H. J., *Brit. Med. Bull.*, **17**: 137-143. 1961.
- 12) SMITH, P. B., *Biochim. Biophys. Acta*, **628**: 19-25. 1980.
- 13) McCANCE, R. A. and E. M. WIDDOWSON, *J. Physiol.*, **147**: 124-134. 1959.
- 14) SUGDEN, M. C., S. C. SHARPLES and P. J. RANDLE, *Biochem. J.*, **160**: 817-819. 1976.
- 15) SEGEL, L. D., A. CHUNG, D. T. MASON and E. A. AMSTERDAM, *Am. J. Physiol.*, **229**: 398-401. 1975.
- 16) BOCKMAN, E. L., D. K. MEYER and F. A. PURDY, *Am. J. Physiol.*, **221**: 383-387. 1971.
- 17) GARTHWAITE, S. M., R. F. MORGAN and D. K. MEYER, *Proc. Soc. Exp. Biol. Med.*, **160**: 401-404. 1979.
- 18) JAYARAM, V., D. C. S. REDDY and B. P. NAIDU, *Experientia*, **34**: 1184-1185. 1978.
- 19) DOLE, V. P., *J. Clin. Invest.*, **35**: 150-154. 1956.
- 20) FULLER, R. W. and E. R. DILLER, *Metabolism*, **19**: 226-229. 1970.
- 21) TANABE, S. and K. KAMEOKA, *Br. J. Nutr.*, **36**: 47-59. 1976.

絶食が数種の哺乳類の心筋、骨格筋および肝臓の グリコーゲン含量に及ぼす影響

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心筋、骨格筋および肝臓のグリコーゲン含量に及ぼす短期間の絶食の影響を、哺乳子山羊、哺乳子豚、ラット、ハムスター、モルモットについて検討した。1) 心筋グリコーゲン；1日1回午前9時に哺乳した子山羊では、哺乳2時間後に最大(8.72 mg/g)となったが、12時間明暗交代下、明期の初期2時間の制限給餌に馴致したラットでは、食餌後有意($P<0.05$)に低下した。自由採食させたラット、ハムスター、モルモットおよび自由哺乳子豚では、採食あるいは哺乳中に低く、絶食により増加した。2) 骨格筋グリコーゲン；哺乳子山羊、制限給餌馴致ラットでは、哺乳あるいは食餌後に増加し、絶食により減少した。自由採食させた動物でも絶食により飽食時の41ないし67%に低下した。また、2.5週齢の子豚では、哺乳中21.4 mg/gと他の動物の4ないし10倍量

含有されており、絶食24時間後も14.3 mg/gと高含量であった。3) 肝臓グリコーゲン；自由採食させた動物では24ないし48時間の絶食により、飽食時の10%以下に減少した。哺乳子山羊、制限給餌ラットでも骨格筋と同様の変化を示した。4) 血漿遊離脂肪酸；子山羊、制限給餌馴致ラット、子豚について測定したが、いずれも哺乳あるいは食餌直後に低下し、絶食により増加した。心筋グリコーゲンとの相関は子山羊では認められなかった。5) 骨格筋と肝臓のグリコーゲンは食餌後高く、食餌前に低い日内変動を示すことが明らかとなったが、心筋グリコーゲンの日内変動は、給餌時刻により変化することが示唆された。

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