Effect of fasting on skeletal muscle triglyceride content

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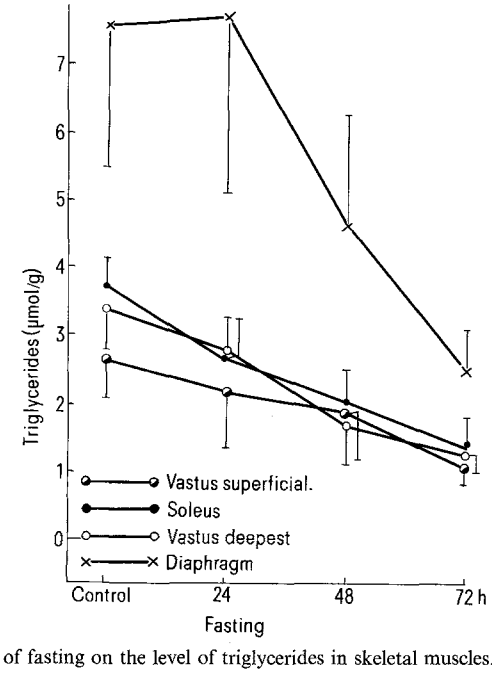
Summary. The effect of prolonged food deprivation on the triglyceride level in different types of skeletal muscle was studied in the rat. It has been found that fasting gradually reduces the triglyceride content in each muscle type. It is concluded that i.m. triglycerides play an important role as energy fuel during fasting.
Key words. Triglycerides; skeletal muscles; fasting, rat.

Skeletal muscles contain considerable amounts of neutral fat[4,9,10,14,22]. It has been consistently shown that this energy store is utilized during prolonged muscular exercise[4-6,10,11,16,20,21,25]. There is no conclusive evidence showing whether skeletal muscle triglycerides (TG) are utilized during fasting. Masoro found the TG level in the gastrocnemuus stable during two days of fasting and elevated on the 3rd day[14]. We have shown that 24-h fasting reduced TG level only in a muscle composed of slow-twitch-oxidative fibers[21]. Therefore, it was the aim of the present study to examine the effect of prolonged food deprivation on the TG content in skeletal muscles composed of different fiber types in the rat.

Methods. Male Wistar rats of 220–260 g b.wt, fed ad libitum on a commercial pellet diet for rodents, were used. A constant light schedule (light on 06.00–18.00 h) was maintained in the animal room. The determinations were carried out in fed rats (control group) and in rats which had fasted for 24, 48 and 72 h. The fasting rats had free access to tap water. The animals were anesthetized with urethane administered i.p. and samples of the following muscles were taken: 1) the superficial layer of the vastus lateralis, 2) the deepest layer of the same muscle, 3) the soleus, 4) the sternal part of the diaphragm. The leg muscle samples have different fiber compositions: the first is composed mostly of fast-twitch-glycolytic fibers (FG muscle), the 2nd of fast-twitch-oxidative-glycolytic fibers (FOG muscle) and the 3rd of slow-twitch-oxidative fibers (SO muscle)[3,18].

Triglycerides were extracted according to the method of Carlson[7] and then quantitatively assayed according to the method of Galletti[12]. The results obtained are presented as means ± SD. Mean values were calculated from the data obtained in 10 rats. The Student t-test for unpaired data was used for statistical evaluation of the results.



Effect of fasting on the level of triglycerides in skeletal muscles.

Results. The levels of TG are presented in the figure. 24 h fasting decreased TG level in FOG ($p < 0.05$) and SO muscle ($p < 0.01$). Fasting lasting for 48 h resulted in further reduction in TG levels in these muscles ($p < 0.001$ and $p < 0.02$ respectively, as compared to the values after 24 h) and reduction in TG level in FG muscle and in the diaphragm ($p < 0.05$ and $p < 0.01$ vs respective controls). 72 h fasting brought about only insignificant further reduction in TG level in FOG and SO muscles. In FG muscle and in the diaphragm TG level was significantly lower than after 48 h ($p < 0.01$ and $p < 0.02$ respectively).

Discussion. The data obtained show that prolonged food deprivation causes gradual reduction in the TG levels in skeletal muscles. It is important to note that this happened also in FG muscle. FG muscle was shown previously to be the only muscle type in which TG were not mobilized during muscular exercise[4,16,20-22]. In the present work it was found for the first time that TG in FG muscle can also be mobilized, despite the low oxidative capacity of this muscle type. Much more TG (in μmoles/g) was mobilized in the diaphragm than in the leg muscles on the 2nd and 3rd days of fasting. This might be caused by the continuous contractile activity of the diaphragm, and thus by greater requirements for energy fuel than in the leg muscles under the experimental conditions described. Other authors found contradictory data on the diaphragm TG content during fasting[1,14,15]. Our results on the TG level in the leg muscles during fasting are in striking contrast to those obtained by Masoro[14]. However, the latter data are very difficult to explain, since, even for the aged rats used in that work, there is no reasonable cause for the marked rise in the muscle TG content observed on the 3rd day of fasting.

An enzyme responsible for hydrolysis of the i.m. TG has not been finally identified, as yet. Recently, strong evidence has been presented that it is the intracellular form of lipoprotein lipase, the extracellular form of which hydrolyses plasma TG[16,17]. There are no data on the regulation of the activity of this intracellular form of the enzyme, except that it is elevated by adrenaline[17]. Isoproterenol, adrenaline and growth hormone activate lipolysis in the diaphragm muscle in vitro and the effect of the former is inhibited by insulin[2,13,19,23]. Blockade of the beta-adrenergic receptors prevents TG mobilization in FOG muscle during exercise[22]. Thus, it seems very likely that the intracellular lipase is under hormonal control. Hypoglycemia developing during fasting changes the humoral balance with increased secretion of growth hormone and glucagon, activation of the adrenergic system and pituitary adrenal axis and decreased secretion of insulin[24]. Such hormonal changes are probably responsible for the activation of lipolysis of the i.m. TG during prolonged food deprivation.

Skeletal muscles comprise more than 40% of total b.wt[8]. It means that the total amount of muscle TG utilized during fasting is substantial. Thus it can be concluded that i.m. TG plays an important role as an energy fuel during fasting.

1 Abumrad, N.A., Stearns, S.B., Tepperman, H.M., and Tepperman, J., J. Lipid Res. *19* (1978) 423.
2 Abumrad, N.A., Tepperman, H.M., and Tepperman, J., J. Lipid Res. *21* (1980) 149.

3 Baldwin, K.M., Klinkerfuss, G.H., Trejung, R.L., Mole, P.A., and Holloszy, H.O., Am. J. Physiol. *222* (1972) 373.
4 Baldwin, K.M., Reitman, J.S., Terjung, R.L., Winder, W.W., and Holloszy, J.O., Am. J. Physiol. *225* (1973) 1045.
5 Barcley, J.K., and Stainsby, W.N., Am. J. Physiol. *223* (1972) 115.
6 Carlson, L.A., Ekelund, L.G., and Fröberg, S.O., Eur. J. clin. Invest. *1* (1971) 248.
7 Carlson, L.A., J. Atheroscler. Res. *3* (1963) 334.
8 Caster, W.C., Poncelet, J., Simon, A.B., and Armstrong, W.D., Proc. Soc. exp. Biol. Med. *91* (1956) 122.
9 Frayn, K.N., and Maycock, P.F., J. Lipid Res. *21* (1980) 139.
10 Fröberg, S.O., Metabolism *210* (1971) 714.
11 Fröberg, S.O., and Mossfeldt, F., Acta physiol. scand. *82* (1971) 167.
12 Galletti, F., Clinica chim. Acta *15* (1967) 124.
13 Garland, F.B., and Randle, P.J., Nature *199* (1963) 381.
14 Masoro, E.J., J. biol. Chem. *242* (1967) 1111.
15 Naptune, E.M., Sudduth, H.C., Jr, Foreman, D.R., and Fash, E.J., J. Lipid Res. *1* (1959) 3.
16 Oscai, B., Caruso, R.A., and Wergeles, A.C., J. appl. Physiol.: Respirat. envir. Exercise Physiol. *52* (1982) 1059.
17 Oscai, L.B., and Palmer, W.K., Exerc. Sport Sci. Rev. *11* (1983) 1.
18 Peter, J.B., Barnard, R.J., Edgerton, V.R., and Gillespie, C.A., Biochemistry *11* (1972) 2627.
19 Randle, P.J., Garland, P.B., Hales, C.N., and Newsholme, E.A., Lancet *1* (1963) 785.
20 Reitman, J.S., Baldwin, K.M., and Holloszy, J.O., Proc. Soc. exp. Biol. Med. *142* (1973) 628.
21 Stankiewicz-Choroszucha, B., and Górski, J., Eur. J. appl. Physiol. *40* (1978) 27.
22 Stankiewicz-Choroszucha, B., and Górski, J., Experientia *34* (1978) 357.
23 Stearns, S.B., Tepperman, H.M., and Tepperman, J., J. Lipid Res. *20* (1979) 654.
24 Tepperman, J., Metabolic and Endocrine Physiology. Year Book Medical Publishers. Inc. (1982).
25 Therriault, D.G., Beller, G.A., Smoke, J.A., and Hartley, L.H., J. Lipid Res. *14* (1973) 54.

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Effects of gastric secretagogues on tissue glycerol in the isolated amphibian gastric mucosa[1]

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Summary. Histamine and theophylline, two gastric secretagogues, significantly increased tissue glycerol content by 121 and 66%, respectively, in the isolated toad gastric mucosa. This is new evidence in favor of the hypothesis that gastric segretagogues may act via lipid mobilization.
Key words. Gastric metabolism; acid secretion; histamine; theophylline; glycerol.

The process of acid secretion by the gastric mucosa is highly dependent on oxidative metabolism. Stimulation of H^+ secretion by gastric secretagogues, such as histamine and theophylline, is associated with an increase in oxygen consumption and substrate oxidation[3-9]. Some lines of evidence in the amphibian gastric mucosa have led to the hypothesis that gastric secretagogues may act via substrate mobilization and that the substrate mobilized is primarily lipid[10-13]. In the present work, we add further evidence in favor of the above hypothesis. If gastric secretagogues mobilize lipids (triglycerides), releasing fatty acids, an increase in the tissue concentration of free glycerol could be demonstrated. Therefore, the effects of gastric stimulants on mucosal glycerol concentration were investigated in the toad gastric mucosa in vitro.

Material and methods. Experiments were performed on gastric mucosa from fasted Venezuelan toads *(Bufo marinus)*. After the toad had been pithed, the stomach was removed and the muscularis layer was stripped from the gastric mucosa and discarded. Paired slices of gastric mucosa, previously weighed, were placed in screw capped 25-ml Erlenmeyer flasks with 5 ml of a Ringer solution containing 17 mM N-tris (hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) at pH 7.4 as a buffer and the testing compounds (10 mM theophylline or 0.1 mM histamine). After gassing with 100% O_2 for 10 min, the flasks were sealed and incubated for 2 h at 30 °C in a shaking water bath. At the end of incubation, the tissues were quickly frozen in liquid nitrogen and immediately pulverized with a stainless steel percussion mortar previously cooled in dry ice. Subsequently, tissue glycerol was extracted by perchloric acid and determined enzymatically with glycerokinase and α-glycerophosphate dehydrogenase, according to the method of Wieland[14]. The amount of reduced nicotinamide adenine dinucleotide (NADH) formed was measured in a Beckman spectrophotometer at 366 nm. Recovery of glycerol in tissue samples was above 95%. The statistical significance of the differences was calculated using Student's t-test.

Results and discussion. The effects of 10 mM theophylline and 0.1 mM histamine on tissue glycerol concentration are shown in the table. These doses of stimulants are known to produce maximal respiratory and secretory responses in the amphibian gastric mucosa in vitro. It can be observed that both gastric secretagogues significantly increased the tissue glycerol concentration above control values, the effect being more pronounced in the case of histamine. The percentile increments were 66 and 121% for theophylline and histamine, respectively.

The present results represent a new evidence in favor of the hypothesis that gastric secretagogues may act via substrate mobilization and that the substrate mobilized is primarily lipid in the isolated amphibian gastric mucosa. This is consistent with a number of previous observations[7,8,10-13]. Alonso et al.[10] have demonstrated a significant decrease in the triglyceride content of the mucosa in association with acid secretion. Assuming that the main sources of free glycerol in the tissue are

Effects of theophylline and histamine on glycerol concentration in the toad gastric mucosa

Condition (n)	Tissue glycerol (μmoles/g wet wt)	%Δ
Control (8)	0.32 ± 0.04	+ 66%*
10 mM theophylline (8)	0.53 ± 0.05	
Control (8)	0.38 ± 0.07	
0.1 mM histamine (8)	0.84 ± 0.09	+ 121%*

Experiments were performed as described in methods. %Δ is the change expressed as percentage of the control values. Values are means ± SE. Numbers in parentheses indicate the number of experiments. * $p < 0.05$ vs control.