Sexually dimorphic response of the mouse submandibular gland to fasting[1]

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Summary. The submandibular gland of male mice contained 18% more DNA, 34% more RNA and 63% more protein than that of female mice. After a 48-h fasting, the percent loss of gland weight, protein, RNA and DNA was greater in the female than in the male.

The sexual dimorphism of submandibular gland in the mouse is exhibited in differences in the cytological differentiation of the tubular portion of the gland[2,3], which has been shown to produce various polypeptides such as epidermal growth factor[4], nerve growth factor[5], renin[6], peptide hydrolases[7], kallikrein[8] and even amylase[9]. The sex difference is also manifested in differences in oxygen consumption, glucose uptake, aerobic glycolysis and adenosine triphosphate production of the gland[10-12]. However, it has not been documented if the sexual dimorphism is also reflected in the chemical composition of the gland. On the other hand, the effect of deprivation of food on the submandibular gland has been investigated in the rat[13], but no comparable studies are available in the mouse. The aim of this study was to investigate, in terms of changes in gland weight and the concentrations and contents of DNA, RNA and protein, a) how the glands of males differ from those of females, and b) whether or not the glands from different sexes respond differently to fasting.

Materials and methods. 10 male and 10 female CF-1 mice (Carworth Branch, Charles River Breeding Laboratories, North Wilmington, Mass.) of 13 weeks of age were used. Food was withheld for 48 h from 5 male and 5 female mice that had free access to water. Remaining 5 male and 5 female mice were given laboratory Purina Chow and water ad libitum. The animals were weighed at the beginning of the experiment and then every 24 h. At the end of the experiment, the animals were killed under ether anesthesia. The submandibular glands were dissected free of the sublingual glands and the connective tissue and weighed. Chemical analyses for DNA, RNA and protein concentrations (mg per 100 mg tissue) of the glands were performed. The DNA content was measured as described previously by Barka[14]. The RNA content was determined using the method of Lin and Schjeide[15]. Deoxyadenosine (1 mg = 2.6 mg DNA) and adenosine-5-phosphate (1 mg = 1.9 mg RNA) were used as standards[16]. The protein was measured by the method of Lowry et al.[17] using human albumin as standard. Since there were 2 variables: sex and food, and 5 animals in each group, 2-way analysis of variance with 5 observations per cell was carried out, using a Hewlett-Packard program on Calculator HP9815. Student's t-test was used when 2 groups of data were compared.

Results and discussion. The male mouse was, on the average, one-fifth heavier than the female mouse and the submandibular gland of the male mouse was one-third heavier than that of the female (table 1). The male gland contained 18% more DNA, 34% more RNA and 63% more protein than the female gland (table 2). All of these differences are significant ($p < 0.01$). Hence, there were about 18% more cells in the male gland than in the female. The higher content of RNA and particularly of protein in the male gland appears to be in accord with the morphological data that the ratio of granular convoluted tubules to acini in terms of areas in the mouse submandibular gland was 1.54 for the male and 0.57 for the female[18]. The granular convoluted tubule cells have been shown to contain various polypeptides as described previously. Thus it is not a surprise to find that the higher concentration of protein (table 2) and the greater protein:DNA ratio (table 3) in the male gland than in the female.

Although the concentration of RNA in the gland was similar between the sexes, the concentration of DNA was higher in the female gland than in the male (table 2). This

Table 1. Effect of fasting on the weight of body and submandibular gland of male and female mice

	B.wt (g)/hours of fasting (h)			Gland weight (mg)	Gland weight (mg)/ b.wt (g)
	0	24	48		
Male					
Fed	31.4±1.7	31.5±2.3	30.8±1.3	190.0±28.7	6.17
Fasted	32.6±2.6	26.4±1.8*	24.4±2.1*	159.4±23.6	6.53
Female					
Fed	25.0±1.7	24.4±2.4	24.4±2.9	121.4±24.0	4.98
Fasted	26.5±1.6	21.8±1.3	19.3±0.7*	84.1±25.3*	4.36

* $p < 0.01$ as compared to the fed control animals by Student's t-test.

Table 2. Effect of fasting on the concentrations and contents of DNA, RNA and protein of submandibular gland of male and female mice

	mg DNA per 100 mg tissue	Total DNA (mg)	mg RNA per 100 mg tissue	Total RNA (mg)	mg protein per 100 mg tissue	Total protein (mg)
Male						
Fed	0.2225±0.0167	0.4202±0.0452	0.7238±0.0932	1.3765±0.2899	16.05±2.33	30.93±9.45
Fasted	0.2609±0.0093*	0.4146±0.0569	0.7267±0.0584	1.1616±0.2187	16.00±2.96	25.53±6.24
Female						
Fed	0.2900±0.0494	0.3434±0.0366	0.7348±0.0368	0.9054±0.1918	9.34±0.67	11.28±2.05
Fasted	0.3353±0.0402	0.2802±0.0796	0.7167±0.0673	0.6001±0.1763**	9.42±1.23	7.96±2.70

* $p < 0.01$; ** $p < 0.05$, as compared to the fed control animals by Student's t-test.

indicates that more cells were present per unit area of the gland in the female and also an average size of cells was smaller in the gland in the female than in the male. The latter is supported by the finding that the RNA:DNA ratio and the protein:DNA ratio of the gland were much lower in the female than in the male (table 3).

The deprivation of food resulted in a comparable loss of body weight in adult mice of both sexes: 18–19% after 24 h and 25–27% after 48 h (table 1). In the adult male rat, a 25% decrease in the b.wt occurred after 72 h of fasting[13]. This probably implies a faster metabolic rate in the mouse than in the rat.

A 48-h fasting resulted in a 16% reduction in the weight of the submandibular gland in the male and a 31% reduction in the female (table 1). Thus, the ratio of gland weight to b.wt after a 48-h fasting was increased in the male but it was decreased in the female.

The loss of gland weight was accompanied by a comparable loss of the total RNA and protein contents of the glands of both male and female mice. In the male, the percent loss of RNA was 16% and that of protein 18%, while in the female, the former was 34% and the latter 29%. The concentrations of RNA and protein, however, were essentially similar in both sexes before and after fasting (table 2).

The greater biochemical changes in the female gland after fasting may be related to the findings that the submandibular gland of female mice contains a greater proportion of acinar cells than ductal cells[2,3,18] and that total inanition affects more the acinar portion than the ductal portion of the salivary gland[13]. It is not known if the cellular turnover rates of RNA and protein in the gland vary between different types of cells or between glands of different sexes. It has been shown, however, that cells from the female gland had a greater oxygen consumption, glucose uptake, aerobic glycolysis and adenosine triphosphate production than cells from the male gland[10–12]; fasting affects these energy-dependent biochemical processes preferentially.

Besides, the concentration of DNA in the gland was significantly ($p < 0.01$) increased following fasting, particularly in the male mice. This was probably secondary to a loss of some cellular components including RNA, protein and water among others. On the other hand, after a 48-h fasting, the total DNA content did not seem to change in the male, but was decreased 18% in the female. This suggests that some cell loss might have occurred as well in the female gland after fasting.

Table 3. Effect of fasting on the RNA:DNA ratio and the protein:DNA ratio in the submandibular gland of male and female mice

	RNA:DNA	Protein:DNA
Male		
Fed	3.28	73.6
Fasted	2.80	61.6
Female		
Fed	2.64	32.8
Fasted	2.14	28.4

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Movements of supernumerary hindlimbs after innervation by single lumbar spinal nerves of *Xenopus laevis*

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Summary. Lumbar spinal nerves S8, S9, and S10, together innervating normal hindlimbs in *Xenopus laevis*, were tested to cause coordinated movements in grafted hindlimbs. It could be shown that this ability is mainly restricted to lumbar nerve S9.

Grafted hindlimbs of the anuran *Xenopus laevis* can only move in coordination and apparent synchrony with the adjacent normal hindlimb (phenomenon of homologous response[3] when innervated by branches of lumbar spinal nerves[4,5]. The same results apply to urodelen amphibia, i.e. *Ambystoma*[6]. Since a hindlimb of *Xenopus* is innervated by the spinal nerves S8, S9, and sometimes S10, the question arises, whether one of these 3 lumbar spinal nerves alone is able to cause adequate limb movement.

Material and methods. Tadpoles of the African clawed toad *Xenopus laevis* were obtained according to the method described by Andres et al.[7]. Autoplastic transplantations of left hindlimbs adjacent to the normal right hindlimb were performed on tadpoles at development stage 54[8]. The animals were anaesthetized in MS 222 and operated on in Holtfreter solution. One of the lumbar nerves, either S8, S9 or S10 (each in 8 tadpoles) was dissected as closely as possible to the base of the right hindlimb. The central stump of the specific lumbar nerve was deviated to the graft's plane of amputation. The animals were raised at 22±2 °C. When the tadpoles had reached stages 62–66 (34–63 days after the operation), the motility of grafted left