Effects of Cold Exposure on Weights, Contents and Fatty Acid Synthesis in Liver and White Adipose Tissues in Rats

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Abstract Rats were exposed to cold at 1-2°C for 3 or 14 days. Changes were measured in the weights of liver, perirenal and epididymal adipose tissue and in the composition of liver. The ability of these organs and of the subcutaneous fat pad to incorporate [^{14}C] acetic acid were compared. It is demonstrated that the mobilization of different substrates induced by cold does not occur in parallel even in the same organ to say nothing of in different organs and that it is not possible to use just one adipose tissue as representative of the whole body. Body weight of 14 days group decreased over the first 6 days of cold exposure then increased to reach the initial value on the 14th day. Liver weight and its protein content decreased on the 3rd day and returned to the initial value on the 14th day while the glycogen content decreased on the 3rd day but increased to a half of initial value on the 14th day. The weights of epididymal and perirenal adipose tissue decreased on the 3rd day and further decreased on the 14th day. Incorporation of [^{14}C] acetic acid into fatty acid in liver tended to decrease in the cold environment. In perirenal and subcutaneous adipose tissue, incorporation did not change on the 3rd day but increased for perirenal and decreased for subcutaneous adipose tissue on the 14th day. In the epididymal fat pad incorporation decreased on the 3rd day and returned to the initial values on the 14th day.

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We have previously investigated changes in gaseous metabolism and body composition of the cold exposed rat. We reported that cold exposure induces a dramatic change of metabolism with a stable stage appearing on the 4-6th day of exposure, although body fat content was low even when body weight returned to the initial value¹⁾. These changes in the whole body must be a reflection of events in the individual organs.

In general, stress conditions result in the catabolism of protein and glycogen as well as fat^{2,3)}. The consumption and deposition of these substances and their cellular concentrations will in turn influence the synthesis and degradation of fat⁴⁾ and the metabolism of other materials. In recent studies we determined the effects of the same conditions of cold exposure on the relation between glucose and fat metabolism in liver and epididymal fat pad *in vitro*. Cold exposure depressed lipogenesis not only in the liver but also in the epididymal fat pad and the depression was not solely regulated by changes in carbohydrate content because a high level of glucose was in-

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cluded in the incubation medium⁹).

The epididymal fat pad is often used for such studies of white adipose tissue metabolism⁶⁻⁹). While this fat pad is a convenient source, it is so small relative to whole body white adipose tissue that it will not quantitatively influence whole body lipid metabolism¹⁰). Consequently, to extrapolate whole body lipid metabolism from results in epididymal fat pad, changes in the pad must parallel other adipose tissues.

This study was therefore designed to investigate the effects of cold exposure on the weights and contents of liver and a variety of white adipose tissues. We compared the ability of liver and white adipose tissue to incorporate [^{14}C] acetic acid into fatty acid *in vivo* during conditions of cold exposure.

Materials and Methods

Animals and diet: Fifteen male albino rats (Wister strain, about 8 weeks of age) were used and they were fed commercial chow pellets (Oriental Yeast Co. Japan) and water *ad libitum*. The animals were caged individually and weighed every other day.

Cold exposure: Before cold exposure, rats were kept in a controlled environment chamber at a temperature of 25°C and relative humidity of 75% for 1 week and rats were divided into three groups. One group (control group) was used initially, after which the temperature in the chamber was decreased to 1-2°C. Rats were kept for 3 or 14 days in this environment.

[^{14}C] acetic acid injection: Five minutes before the experiment rats were moved from the cold room to the laboratory at about 23°C, and 20 mg of pentobarbital-Na in 1 ml of saline was injected by hypodermic syringe to induce anesthesia. After 15 minutes, [^{14}C] acetic acid (8.8 μCi , sodium salt, 45-60 mCi/mmol, New England Nuclear, USA) was injected rapidly through the *venae femoralis*. Fifteen minutes after the injection, rats were killed by exsanguination through the *vena cave caudalis*. The liver, epididymal and perirenal fat pads were excised and weighed, and the subcutaneous adipose tissue with skin and fur was flayed from the muscle just above the hip joint. Tissues were divided into 1 g portions for liver, 0.5 g for adipose tissues and subcutaneous adipose tissue was cut into 3 cm squares. They were frozen rapidly in dry ice and methanol and stored at -20°C. These procedures were done at 9-12 a. m.

Analytical methods: One gram of liver was homogenized to extract total lipid by BLIGH and DYER's method¹¹). The chloroform solution containing total lipid was transferred to a weighed test tube, dried and reweighed to measure the total lipid content in liver. The lipid was dissolved again in 10 ml of chloroform: methanol (2:1) and 2 ml of water was added. After centrifugation (1500 g, 10 min) the upper phase was removed, and the lower phase was rinsed with 4 ml of methanol: water (1:0.9) mixture to eliminate the radioactive substrate. The solution was transferred into a counting vial and dried, and 10 ml of scintillation solution (0.5% of PPO and 0.01% of POPOP (Dojin Lab.) in toluene) was added and the incorporation of ^{14}C radioactivity into the total lipid determined.

The method for the measurement of incorporation of $[U-^{14}C]$ acetic acid into fatty acid in liver and adipose tissues was the same as in previous work⁵⁾.

The glycogen content of liver was measured by an anthrone method¹²⁾. Plasma non-esterified-fatty-acid (NEFA) was determined using a colorimetric procedure (Wako test kit NEFA test; Wako Pure Chemical Industries, Japan), based on the method of ITAYA and U¹³⁾ and glucose was measured by the glucose oxidase method of HUGGEST and NIXON¹⁴⁾.

Calculation: Incorporation of radioactivity was corrected for differences in body weight (BW.) using the formula below $DPM \text{ corrected} = (BW. \text{ in grams}/100 \text{ g}) \times (5 \mu\text{Ci}/8.8 \mu\text{Ci}) \times (DPM \text{ measured})$ to correspond to $5 \mu\text{Ci}/100 \text{ gBW.}$

Data were analysed using MANN-WHITNEY'S U test, because some of the distributions of data were not normal and differed between groups¹⁵⁾.

Results

Changes in body weight of the 14 day group are illustrated in Fig 1. Weight increased by 2.3 g per day and reached $207 \pm 8 \text{ g}$ at 25°C . Over the first 6 days of cold exposure it decreased by 17 g, then increased by 2.0 g per day and on the last day of cold exposure it returned to the initial body weight.

Weights and compositions of organs are summarized in Table 1. Liver weight decreased on the 3rd day and returned to control levels on the 14th day, but body weight changed in the same manner, so that the proportion of liver weight to body weight did not change. Glycogen content decreased sharply on the 3rd day and increased on the 14th day but did not reach the control value. Fat content did not change but protein content significantly decreased on the 3rd day and recovered on the 14th day.

The absolute weights and weights relative to body weight of the epididymal and

Fig. 1. Body weight changes of rats ($n=5$) exposed at $1-2^\circ\text{C}$ for 2 weeks and fed *ad libitum*. Values given as means $\pm$ SD.

Table 1. Weight and composition of cold (1-2°C) exposed rats

	Control	Days in cold	
		3	14
Body weight (g)	212.3 ± 10.6	202.6 ± 10.1	208.4 ± 17.3
Liver weight (g)	8.29 ^a ± 0.40	7.62 ^b ± 0.56	8.32 ^{a,b} ± 0.78
Proportion of body weight (%)	3.91 ± 0.24	3.76 ± 0.22	3.99 ± 0.16
Glycogen (%)	3.72 ^a ± 0.29	0.400 ^b ± 0.346	1.97 ^c ± 0.50
Lipid (%)	4.41 ± 0.24	4.38 ± 0.39	4.36 ± 0.37
Protein (%)	15.3 ^a ± 0.78	14.2 ^b ± 0.99	14.9 ^{a,b} ± 0.33
Epididymal fat pad weight (g)	2.28 ^a ± 0.20	2.11 ^a ± 0.27	1.19 ^b ± 0.38
Proportion of body weight (%)	1.06 ^a ± 0.14	1.04 ± 0.10	0.565 ^b ± 0.154
Protein (%)	2.91 ± 0.50	2.61 ± 0.52	2.42 ± 0.49
Perirenal adipose tissue weight (g)	1.62 ^a ± 0.277	1.36 ^{a,b} ± 0.47	0.876 ^b ± 0.462
Proportion of body weight (%)	0.765 ^a ± 0.131	0.681 ^{a,b} ± 0.264	0.414 ^b ± 0.203
Protein (%)	2.09 ^a ± 0.36	1.99 ^a ± 0.49	3.33 ^b ± 0.66

Values are expressed as means and SD (n=5). a, b: Mean values in the same horizontal row having different superscript letters are significantly different from one another (P < 0.05).

Table 2. Effect of cold exposure on plasma glucose and non-esterified fatty acid (NEFA) concentration

	Control	Days in cold	
		3	14
Plasma glucose (mg/dl)	129 ^a ± 12	148 ^b ± 12	122 ^a ± 11
Plasma NEFA (mEq/l)	0.238 ^a ± 0.101	0.337 ^b ± 0.042	0.320 ^{a,b} ± 0.129

Values are expressed as means and SD (n=5). a, b: Mean values in the same horizontal row having different superscript letters are significantly different from one another (P < 0.05).

Table 3. Effects of 3 and 14 day cold exposure on the incorporation of [U-¹⁴C] acetic acid into total lipid and fatty acid by rat liver

	Control	Days in cold	
		3	14
Into total lipid DPM*/g tissue	0.271 ± 0.084	0.199 ± 0.088	0.203 ± 0.073
DPM/mg protein	170 ± 54	137 ± 56	137 ± 49
DPM*/whole liver	2.24 ± 0.76	1.51 ± 0.68	1.71 ± 0.68
Into fatty acid DPM*/g tissue	0.214 ± 0.106	0.170 ± 0.051	0.190 ± 0.065
DPM/mg protein	140 ± 69	118 ± 30	127 ± 44
DPM*/whole liver	1.78 ± 0.91	1.29 ± 0.42	1.58 ± 0.53
(Fatty acid/Total lipid: DPM/mg protein) × 100 (%)**	91 ± 17	93 ± 23	95 ± 12

Values are expressed as means and SD (n=5). *: Incorporation of radioactivity is expressed as 10⁻⁵ DPM. **: The percentage of the incorporation of [U-¹⁴C] acetic acid into fatty acid to that of into total lipid.

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Table 4. Effects of 3 and 14 days cold exposure on the incorporation of [U-¹⁴C] acetic acid into fatty acid by rat adipose tissues

	Control	Days in cold	
		3	14
Epididymal fat pad DPM/g	5.45 ± 2.75	2.83 ± 2.35	6.82 ± 5.77
DPM/mg protein	0.193 ± 0.087	0.103 ± 0.079	0.303 ± 0.279
Total	13.1 ± 0.73	6.03 ± 4.75	8.73 ± 9.16
Perirenal adipose tissue DPM/g	4.50 ^a ± 4.09	4.77 ^{a,b} ± 2.52	40.1 ^a ± 31.7
DPM/mg protein	0.223 ± 0.210	0.253 ± 0.149	1.29 ± 1.04
Total	7.46 ± 6.88	6.91 ± 4.98	39.2 ± 39.7
Subcutaneous adipose tissue DPM/cm ²	2.59 ^a ± 0.72	2.69 ^a ± 0.80	1.65 ^b ± 0.39

Values are expressed as means and SD (n=5). Incorporation of radioactivity is expressed 10⁻³ DPM. a, b: Mean values in the same horizontal row having different superscript letters are significantly different from one another (P < 0.05).

perirenal adipose tissues decreased on the 3rd day and in contrast with liver they decreased further on the 14th day. The protein content of epididymal fat pad tended to decrease gradually but that of perirenal adipose tissue did not change on the 3rd day and increased on the 14th day.

Plasma glucose concentration increased on the 3rd day but returned to the control value on the 14th day. Plasma NEFA concentration increased on the 3rd day and elevated levels were maintained on the 14th day (Table 2).

The incorporation of [U-¹⁴C] acetic acid into total lipid in the liver (Table 3) tended to decrease in the cold but there was no difference between the 3 and 14 day groups. The incorporation of [U-¹⁴C] acetic acid into fatty acid tended to decrease in the 3 day group and to increase on the 14th day but these changes were not significant (P < 0.05). The proportion of incorporation into fatty acid to that into total lipid was not affected by cold exposure.

In the epididymal fat pad the incorporation of [U-¹⁴C] acetic acid decreased to a half of the control value (P < 0.08) by day 3 and returned to the control value on the 14th day. In the perirenal adipose tissue, incorporation did not change on the 3rd day but it increased to about ten times the control value on the 14th day. In the subcutaneous adipose tissue incorporation did not change on the 3rd day and decreased on the 14th day (Table 4).

Discussion

1. *Body weight:* We have previously demonstrated that when the rat was exposed to cold (1-2°C) it could increase heat production immediately but it took about 1 week to increase food intake¹⁾. The shift of body weight, with a temporary decrease and subsequent recovery (Fig. 1), may be due to this time lag. This change of body weight indicates that metabolism of the rats used on the 3rd day was catabolic and that on the 14th day was anabolic. These metabolic changes in the whole body will have a profound influence on the weight, composition and rate of fatty acid

synthesis in each organ of the body.

2. *Weights and compositions of organs*: A notable finding from the results for liver is that a clear mobilization of liver protein as well as of glycogen was demonstrated. It is well known that not only cold exposure but also stresses in general induce glycogen breakdown and release of glucose from the liver¹⁶. A temporary increase and recovery of plasma glucose concentration agrees with the decrease of glycogen on the 3rd day and an increase on the 14th day. Those shifts further reflect the time course of changes in whole body energy balance and adaptation to cold stress¹¹.

In this experiment, not only did glycogen and protein contents decrease but the weight of the liver also decreased. Consequently it is clear that liver protein is mobilized on a large scale and that the liver is important for preservation and supplementation of protein supplies in acute cold exposure. It has been reported that cold stress induces an accelerated turnover of protein and increased nitrogen excretion^{9,17,18}.

By the 14th day liver weight and protein content had recovered, while glycogen content was still somewhat depressed. These results indicate that in the time course of adaptation to cold, mobilization and regeneration of different tissue components do not always occur in parallel to each other, even in the same organ and this tendency is clearer when different organs are compared.

The characteristic changes in adipose tissue is that in contrast with liver, the weights of the adipose tissues did not parallel changes in body weight, but continued to decrease even when energy balance became positive. The plasma NEFA concentration did not return to the control value but remained high on the 14th day. These results suggest that mobilization of fat continued and that the proportion of fat used as an energy source was high even in the latter part of the cold exposure. We have previously exposed rats to cold and observed changes in the respiratory quotient, which was below control values on the 11th day of a cold exposure¹¹. In that study, as in the present work the body fat content remained depressed when body weight had returned to the control values¹¹.

Comparison of the results for adipose tissue with those for liver indicate that acute cold exposure induces mobilization of substrates used as energy or carbon sources but mobilization of different substrates does not always parallel whole body energy balance nor does mobilization of different substrates parallel each other, even in the same organ to say nothing of in different organs.

3. [U - ^{14}C] *acetic acid incorporation into fatty acid*: It is well known that cold exposure changes lipid metabolism in liver¹⁹ and white or brown adipose tissues^{9,10}. We established that changes in fatty acid synthesis differ between liver and epididymal fat pad from cold exposed rats *in vitro*⁹. The conclusion of the present work is that changes in fatty acid synthesis differ between white adipose tissues from different parts of same rats.

Measurement of lipogenesis using ^{14}C -labeled substrates may be less satisfactory than using 3H_2O because the concentration of the former may differ between different organs²⁰. Therefore we wish to discuss not comparisons between absolute values in

the various organs but rather the shifts in metabolism from the control values.

The changes in incorporation of [U- 14 C] acetic acid into total lipid or fatty acid in liver were not clear but the tendency for incorporation to decrease agrees with previous work⁵⁾ and other reports^{2,10,19,20,22)}. Further experiments using more animals will be required to confirm this tendency.

In epididymal fat pad the decreased incorporation of [U- 14 C] acetic acid into fatty acid was not significant ($P < 0.05$). Since this trend agrees with the previous experiment *in vitro*⁵⁾ and the conventional significance level was approached ($P < 0.08$), fatty acid synthesis in this organ might parallel whole body energy balance.

In contrast with the result for epididymal fat pad, the incorporation in perirenal adipose tissue did not change on the 3rd day but increased to ten times the control value on the 14th day. The variability of the 14th day values was great but the lowest incorporation recorded on the 14th day was higher than the greatest value on the 3rd day. A different pattern of incorporation was apparent in the subcutaneous adipose tissue, where incorporation did not change on the 3rd day as in the perirenal adipose tissue, but in contrast with the latter it decreased on the 14th day. Since these tissues were obtained from same animal it can be concluded that the effects of cold exposure differ between white adipose tissues in different parts of the body. These results indicate that epididymal fat pad, which is often used to investigate white adipose tissue, does not always represent all white adipose tissues.

Since the subcutaneous depot is the largest of the body fat stores, changes in fatty acid synthesis in this tissue will have a greater influence on whole body lipid metabolism than for other fat depots. The subcutaneous adipose tissue could itself be differentially affected by cold in different regions of the body. Further experimentation is required to explore this possibility.

It is not clear why the effect of cold differs between white adipose tissues. A few observations have been reported which may give some clues as to the mechanisms involved. According to ITO the activity of fatty acid synthesis in adipose tissue decreases as temperature falls and is abolished at 17°C. Insulin, which accelerates fatty acid synthesis, is less effective at low temperatures but fatty acids will still be released by adrenalin or noradrenalin²³⁾. WEEKES *et al.* reported that whole body responsiveness to insulin is enhanced in cold exposure²⁴⁾. These facts may explain the decrease of fatty acid synthesis in epididymal fat pad or subcutaneous adipose tissue and the increase in perirenal adipose tissue because the former two are relatively superficial and may be strongly influenced by environmental temperature and the latter is located deep within the body. It is well known that cold changes the blood flow distribution. In particular flow to subcutaneous adipose tissue would be reduced, so that the supplies of substrate for fatty acid synthesis would be less in this tissue than in the warm.

In this experiment rats were allowed to feed *ad libitum*, inducing an increase of food intake¹⁾ with a greater supply of carbon sources which would dilute 14 C-labeled substrate, and would stimulate an increase secretion of insulin. These factors may

also affect the incorporation of ^{14}C -labeled substrate into fatty acid.

The above considerations still do not identify the major factors which control fatty acid synthesis in individual tissues and do not explain the differences between the results on the 3rd and the 14th day of cold exposure.

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低温暴露がラットの肝臓と白色脂肪組織の重量・成分 および脂肪酸合成におよぼす影響

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ラットを低温 (1~2°C) に暴露し, その3日目と14日目に肝臓の重量・成分および腎周囲と副睾丸脂肪組織の重量さらにこれらの組織と皮下脂肪組織における¹⁴C酢酸の脂肪酸への取込みを比較した。その結果, 低温暴露による異なる物質の動員は, 異なる臓器ではもちろん, 同じ臓器でも並行には進まないこと, 1カ所の脂肪組織を全身の代表として用いることはできないことが示された。

体重は低温暴露前半で低下したが, 後半は増加して14日目には暴露前の値を回復した。肝臓の重量と蛋白質含量は3日目に低下し, 14日目には暴露前の値を回復し

た。一方グリコーゲン含量は3日目に低下し, 14日目には暴露前の値の半分まで増加した。副睾丸脂肪組織と腎周囲脂肪組織の重量は3日目で低下し, 14日目でさらに低下した。

肝臓での¹⁴C酢酸の脂肪酸への取込みは低温で低下の傾向を示した。腎周囲脂肪組織と皮下脂肪組織では3日目では取込みは変化しなかったが, 14日目では腎周囲脂肪組織では増加, 皮下脂肪組織では低下した。副睾丸脂肪組織では取込みは3日目で低下, 14日目で暴露前の値へもどった。

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