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Effect of different experimental conditions on lipogenesis and substrate oxidation in isolated adipocytes from fattening Holstein steers

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Summary – The present study was undertaken to provide further information on lipogenesis in isolated adipocytes from the subcutaneous adipose tissue of fattening steers. The main aims were to compare different incubation media and to clarify the effects of fetal bovine serum (FBS) on lipogenesis and substrate oxidation rate in the adipocytes. The isolated adipocytes were prepared by the collagenase digestion technique. The changes in cellularity, the incorporation rates of acetate or glucose into lipid molecules, and the oxidation rates to CO_2 both in fresh and preincubated adipocytes were measured in different media. It was shown that FBS increased significantly both lipogenesis and the oxidation rates of glucose or acetate in the fresh adipocytes but insulin did not. In isolated adipocytes preincubated for 24 and 48 h, the cellularity, the incorporation rate of glucose into lipid molecules, and the oxidation rates of glucose or acetate to CO_2 were not different to those in fresh adipocytes, but the incorporation rate of acetate into lipid molecules was significantly lower.

adipocytes / lipogenesis / steers / fetal bovine serum / insulin

Résumé – Effets de différentes conditions expérimentales sur la lipogenèse et l'oxydation des substrats dans des adipocytes isolés issus de bœufs Holstein à l'engraissement. Ce travail a été conçu pour étudier la lipogenèse dans des adipocytes d'origine bovine isolés à partir de tissus sous-cutanés. L'objectif majeur est de comparer différents milieux d'incubation, et de clarifier les effets du sérum de veau fœtal sur la lipogenèse et l'oxydation de substrats. Les adipocytes ont été préparés selon la technique de la digestion à la collagénase. L'évolution de la cellularité, la transformation de l'acétate ou du glucose en lipides et du taux d'oxydation en CO_2 dans les adipocytes frais ou pré-incubés ont été mesurées dans différents milieux d'incubation. L'utilisation de l'acétate pour la lipogenèse est supérieure à celle du glucose. En revanche, le sérum de veau fœtal augmente aussi bien la lipogenèse que l'oxydation du glucose et de l'acétate. Cependant, l'insuline n'a pas eu d'effet. Dans les adipocytes

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isolés pré-incubés pendant 24 à 48 h, la transformation du glucose en lipides ainsi que l'oxydation en CO_2 du glucose en acétate ne variaient pas, tandis que la transformation de l'acétate en lipides diminuait fortement.

adipocytes / lipogenèse / bovin / sérum de veau fœtal / insuline

INTRODUCTION

Lipogenesis in the adipose tissue of steers during the fattening period is very important to the energetic and economic efficiency of meat production. Up to now, most studies on lipogenesis in bovine adipose tissue measured lipogenesis by incubating adipose tissue explants for hours or days (Smith et al, 1984; Miller et al, 1991; Chilliard and Faulconnier, 1995; May et al, 1995). The main advantage of this preparation is in the simplicity of tissue sample preparation and the limited manipulation of the tissue. But adipose tissue explants include a variety of cell types including not only adipocytes, but also preadipocytes, endothelial cells, fibroblasts and macrophages (Hausman and Martin, 1987). Following the introduction of the collagenase technique to isolate the adipocytes (Rodbell, 1964) and further modifications of this technique, the use of isolated bovine adipocyte preparations is preferred by some researchers interested in adipose tissue morphology and metabolism, especially in studies on hormone binding to adipocytes (Yang and Baldwin, 1972; Vasilatos et al, 1983; Vernon et al, 1985). There are some possible limitations to this technique such as the possible damage to the cells produced by excessive collagenase exposure, the fragility of the cells and possible release of protease during a prolonged incubation of isolated adipocytes (Hausman and Martin, 1987).

The research concerning lipogenesis of bovine isolated adipocytes, either fresh or following preincubation, is limited. Also, there is no information on the comparison of incubation media such as Medium 199 (M199), which is used extensively in isolated adipocyte culture systems, and Krebs–Ringer bicarbonate buffer (KRB). Recently, it was reported that fetal bovine serum (FBS) changed the metabolic activity of bovine adipocytes and suppressed the effect of insulin on lipoprotein lipase (Faulconnier et al, 1994). In order to compare different incubation media and to clarify the effects of FBS on lipogenesis and substrate oxidation in isolated adipocytes, we prepared isolated adipocytes from the subcutaneous adipose tissue of fattening steers, measured the changes in cellularity, compared the differences in lipogenesis and substrate oxidation rate between fresh and preincubated isolated adipocytes, incubated in different media with, or without, FBS and insulin.

MATERIALS AND METHODS

Animals and tissue sampling

Four Holstein steers, 13 months of age and having body weights ranging from 380 to 420 kg, were fed on a daily diet of 6 kg of concentrate plus 3 kg hay and water ad libitum. The concentrate consisted of: ground corn, 56%; wheat bran, 24%; soybean oil meal, 12%; and required vitamins and salts. Subcutaneous adipose tissue samples were obtained using a biopsy technique from the region of the last thoracic vertebrae, 20 to 30 cm to the right or left of the dorsal midline. As a local anesthetic we used Xylocaine (Lidocaine Hydrochloride, Fujisawa Pharmaceutical Co, Ltd, Osaka, Japan). The tissue was transported to the laboratory in saline at 37 °C within 20 min and then rinsed with fresh saline.

Isolation of adipocytes

The adipocytes were isolated based on a modified method of Rodbell (Yang and Baldwin, 1972; Vasilatos et al, 1983). Samples of adipose tissue approximately 2 g in weight were dissected free of the connective tissue and blood vessels. The tissue was then placed in 25-mL polypropylene beakers containing 6 mL Medium 199 (M199, pH 7.4; GIBCOBRL, Life Technologies, Inc Grand Island, NY, USA) containing Earle salts, 4.2 mM $NaHCO_3$, 5.5 mM glucose and 0.61 mM acetate, and supplemented with 20 mM HEPES, 4.5 mM glucose, 4.39 mM acetate, 4% BSA (bovine serum albumin, fraction V, Sigma Chemical Co, St Louis, MO, USA) and 3 mg per mL collagenase (Type III, Worthing Biochemical Co, New Jersey, USA) (Vernon et al, 1985). After being minced and gassed for 30 s with a 95% O_2–5% CO_2 mix, the tissue was digested in a water bath at 37 °C, vibrating at 60 cycles per min for 90 min. After digestion, the cell suspensions were filtered through a polypropylene mesh with 1 000 μm pore size and washed with warm collagenase-free M199 or KRB (pH 7.4) (Vasilatos et al, 1983) supplemented 20 mM HEPES, 10 mM glucose, 5 mM acetate, 4% BSA. Then, the cells were filtered through a polypropylene mesh with 250 μm pore size and washed three times. This pore size allowed the largest adipocytes to pass without rupturing.

Preliminary incubation

In experiment 1, the medium for the 24-h preincubation was based on the supplemented M199 (pH 7.4), which was the same as the medium used for digestion but was collagenase-free and supplemented with penicillin (100 units/mL), streptomycin (100 μg/mL), neomycin sulfate (10 μg/mL) and amphotericin B (5 μg/mL). In experiment 2, the medium used for both the 24- and 48-h preincubation was based on the supplemented M199 (pH 7.4), which was the same as the medium used in experiment 1, but supplemented with 10% FBS (fetal bovine serum, GIBCOBRL, Life Technologies, Inc, Grand Island, NY, USA). For the adipocyte preincubation we used standard cell culture technique. The isolated adipocytes were plated at low density and floated at the air–liquid interface. Approximately 10^4 cells per mL of adipocyte suspension were transferred to each well of the 24-well culture plates. The wells were 17.6 mm in diameter and 22.1 mm deep. To maintain proper pH and nutrient supply, the media were changed every 6 h.

Lipogenesis and substrate oxidation in vitro

Both fresh and preincubated isolated adipocytes were used for comparing the lipogenesis rate in KRB or M199 with 10 ng/mL insulin (bovine insulin, sodium, Becton Dickinson Labware, Bedford, MA, USA), and with or without 10% FBS. Aliquots of isolated adipocytes (100 μL) were added to the tubes containing 1 mL of the following incubation media: KRB, KRB + insulin, KRB + FBS, M199, M199 + insulin, M199 + FBS, M199 + FBS + insulin in experiment 1 and only M199 + FBS in experiment 2. Tubes containing 1 mL of medium with the same volume of cell aliquots were used to determine the density of cells in the medium for incubation. After 1 μCi D-[U-^{14}C] glucose (specific activity: 283 mCi per mmol, Amersham, UK) or 1 μCi [U-^{14}C] acetic acid, sodium salt (specific activity: 59 mCi per mmol, Amersham, UK) was added into the media. The tubes were then gassed for 1 min with a 95% O_2 : 5% CO_2 mix, capped and incubated for 2 h in a vibrating water bath at 37 °C. At the end of the incubation, to terminate the incubation and liberate the CO_2, 0.25 mL 6 N H_2SO_4 was injected into the tubes. Then the samples were swirled for 60 additional minutes to ensure the liberation of the CO_2 within the medium. CO_2 was trapped by pieces of NaOH paper, which had been in a center well. Then the papers were removed to glass scintillation vials. After standing overnight with 10 mL of a scintillation cocktail (UL TIMA-FLO™M, Packard, USA), the vials were counted in a liquid scintillation counter (LSC-5102, Aloka, Japan). The total amount of lipids was extracted by the method of Dole (Dole, 1956). Samples were evaporated to dryness, resuspended in 10 mL of the scintillation cocktail, and radioactivity was measured. Tubes containing medium with substrate and tracer, but no cells, were included to determine the background values for CO_2 and lipid radioactivity. The incorporation rate of ^{14}C-labeled glucose or acetate into the total amount of lipid material was calculated as nmol glucose or acetate incorporated per 2 h per 10^5 cells. The ^{14}C-CO_2 from the oxidation of ^{14}C-labeled glucose or acetate was calculated as nmol glucose or acetate oxidized per 2 h per 10^5 cells. The cal-

culations were based on a supposition that glucose and acetate are totally (all carbons) oxidized to CO_2. Relative contributions of acetate and glucose to the lipogenesis and oxidation rate in isolated adipocytes were expressed by the ratio of incorporation rate of acetate (A) to the rate of glucose (G) or the ratio of oxidation rate of acetate (A) to the rate of glucose (G). In ruminant adipose tissue, acetate carbon is essentially all incorporated into fatty acid, whereas glucose carbon is mostly incorporated into the glycerol moiety of acylglycerols (Smith and Crouse, 1984).

Adipocyte cellularity

Before the metabolic incorporation experiments, the diameters of fresh and preincubated adipocytes were calculated by a micro photograph method. After being isolated by collagenase digestion or preincubated, the adipocytes together with an objective micrometer (0.01 mm, Nikon, Japan) were immediately photographed using micro photograph equipment (OPTIPHOT, Nikon, Japan). For every treatment more than 300 cells from the photographs were used for measuring the mean diameter and the diameter distribution. For comparison, the mean diameter and the diameter distribution of adipocytes in adipose tissue was also measured using urea to liberate the cells after osmium tetroxide-fixation (Hirsch and Gallian, 1968). Because there was little difference in the diameters and cell number frequency distributions between adipocytes isolated with collagenase and those liberated using urea after osmium tetroxide-fixation (He et al, unpublished), only the diameters of adipocytes isolated with collagenase were used.

Statistical analysis

In experiment 1, a two-way Anova with replication was used as the model to test the differences between the media (M, df = 6) or the time of preincubation (T, df = 1) and their interaction (M × T, df = 6). In experiment 2, a single classification Anova was used as the model to test the differences between the times of preincubation. The Duncan mutiple-range test was used for mutiple comparisons of the means when a significant difference ($P < 0.05$) was found (Littell et al, 1991).

RESULTS

Experiment 1

Cellularity

The adipocyte diameter and cell number frequency distributions from subcutaneous adipose tissue in steers are shown in figure 1a. Although there was a trend towards a lower percentage of large adipocytes after 24 h preincubation, no significant change in the diameter or cell number frequency distributions was found.

The effect of FBS and insulin added into different media on lipogenesis in fresh and 24-h preincubated isolated adipocytes

The incorporation rate of glucose into lipid molecules was generally lower in adipocytes preincubated for 24 h, but there was no significant difference in each pair of means between fresh and preincubated adipocytes (table I). The addition of FBS during the 2-h incubation increased significantly the incorporation rate of glucose into lipid molecules in both fresh and preincubated adipocytes, but insulin did not.

The incorporation of acetate into lipid molecules also was generally lower in adipocytes preincubated for 24 h, compared with the incorporation rate in fresh adipocytes. However, the difference was only significant in cells incubated in media with FBS (table I). The addition of FBS during the 2-h incubation also increased significantly the incorporation rate of acetate into lipid molecules in adipocytes, but insulin did not.

The effect of the addition of FBS and insulin in the different media on the oxidation rate of glucose and acetate to CO_2 in fresh and preincubated isolated adipocytes

The oxidation rate of acetate and glucose to CO_2 was significantly lower in adipocytes

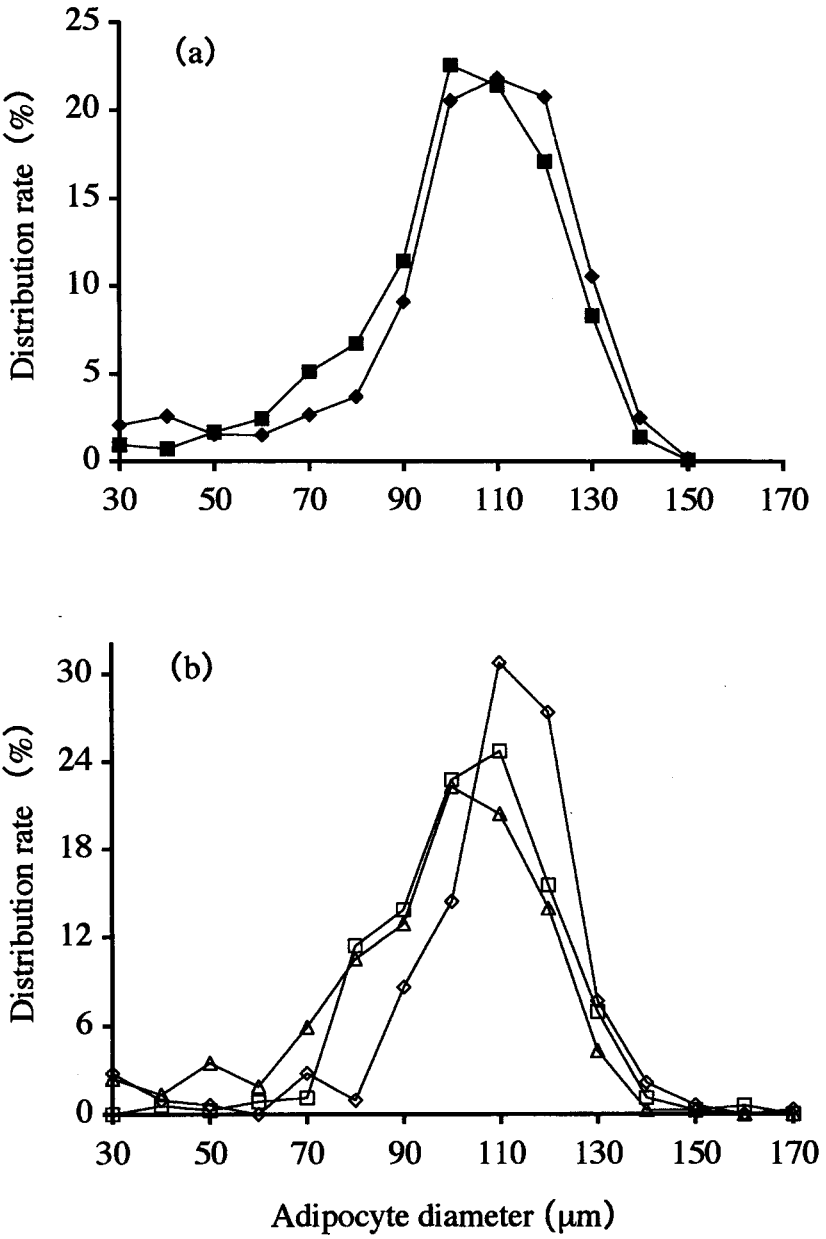

Fig 1. The distribution of cell diameters for fresh isolated adipocytes and those isolated adipocytes preincubated in media with or without FBS, which were used in experiments 2 and 1, respectively. (a) Without FBS: ◆, fresh adipocytes, mean diameter: 107 μm; ■, 24 h preincubated, mean diameter: 106 μm. (b) With FBS: ◇, fresh adipocytes, mean diameter: 105 μm; □, 24 h preincubated, mean diameter: 109 μm; △, 48 h preincubated, mean diameter: 103 μm.

Table I. The effect of media and preincubation on incorporation rates of glucose and acetate into lipid in isolated adipocytes from four fattening steers*.

*Time and medium***	*Glucose to lipid* mean ± se	*Acetate to lipid* mean ± se	*Ratio* A/G**
	fresh adipocytes (nmol/2h/10^5 cells)		
KRB	22.5 ± 3.4 ^{cd}	160 ± 6 ^{e}	7
KRB + insulin	19.9 ± 1.8 ^{cd}	175 ± 5 ^{de}	9
KRB + FBS	35.7 ± 3.8 ^{ab}	921 ± 22 ^{b}	26
M199	15.6 ± 1.8 ^{de}	147 ± 5 ^{e}	9
M199 + insulin	16.2 ± 0.4 ^{de}	148 ± 12 ^{e}	9
M199 + FBS	28.9 ± 2.2 ^{bc}	1172 ± 77 ^{a}	40
M199 + FBS + insulin	30.6 ± 2.9 ^{bc}	1029 ± 79 ^{ab}	34
	24-h preincubated adipocytes (nmol/2 h/10^5 cells)		
KRB	14.1 ± 0.8 ^{de}	117 ± 6 ^{e}	8
KRB + insulin	14.5 ± 0.7 ^{de}	115 ± 2 ^{e}	8
KRB + FBS	44.9 ± 0.8 ^{a}	359 ± 47 ^{d}	8
M199	12.3 ± 3.5 ^{de}	56 ± 16 ^{e}	5
M199 + insulin	8.0 ± 0.6 ^{e}	64 ± 9 ^{e}	8
M199 + FBS	21.7 ± 2.6 ^{cd}	704 ± 19 ^{c}	32
M199 + FBS + insulin	22.6 ± 0.7 ^{cd}	707 ± 52 ^{c}	31
	****	****	

* Experiment 1; medium for preincubation was M199 without FBS; ** ratio of incorporation rate of acetate (A) to the rate of glucose (G); *** both time and medium means differ significantly ($P < 0.05$); **** the interaction between time and medium is significant ($P < 0.05$). $^{a, b, c, d, e}$ Values within a column without the same superscripts differ significantly ($P < 0.05$).

preincubated for 24 h and were then incubated in KRB + FBS (table II).

The addition of FBS into M199 increased significantly the oxidation rate of glucose and acetate to CO_2 in both fresh and preincubated adipocytes, and the addition of FBS into KRB increased significantly the oxidation rate of glucose to CO_2 in fresh adipocytes but did not do so in preincubated adipocytes (table II).

Relative contributions of acetate and glucose to the lipogenesis and oxidation rates in fresh and preincubated isolated adipocytes

As a precursor for lipogenesis, acetate had a larger incorporation rate into lipid molecules than glucose for all the experimental conditions. Ratios of the incorporation rates of acetate into lipid molecules to the rate of glucose were 26, 40 and 34 in fresh adipocytes incubated in KRB + FBS, M199 + FBS and M199 + FBS + insulin, respectively, 7 in KRB, and 9 in KRB + insulin, M199 and M199 + insulin (table I). The ratios became sightly lower in adipocytes preincubated for 24 h, except in KRB + FBS where the ratio decreased sharply owing to the decrease in acetate incorporation. The addition of FBS increased these ratios in all the media except with KRB + FBS for preincubated adipocytes.

Ratios of oxidation rate of acetate to CO_2 to the rate of glucose were 0.3 to 0.6 what-

Table II. The effect of media and preincubation on oxidation rates of glucose and acetate to CO_2 in isolated adipocytes from four fattening steers*.

*Time and medium****	*Glucose to CO_2* *mean ± se*	*Acetate to CO_2* *mean ± se*	*Ratio* *A/G***
	fresh adipocytes (nmol/2h/10^5 cells)		
KRB	2.3 ± 0.1 ^{c}	0.9 ± 0.1 ^{d}	0.4
KRB + insulin	2.7 ± 0.1 ^{c}	1.1 ± 0.1 ^{cd}	0.4
KRB + FBS	12.4 ± 1.1 ^{a}	4.6 ± 0.1 ^{a}	0.4
M199	7.1 ± 2.1 ^{bc}	2.7 ± 0.3 ^{bcd}	0.4
M199 + insulin	4.8 ± 0.8 ^{c}	2.8 ± 0.1 ^{abc}	0.6
M199 + FBS	11.5 ± 0.4 ^{ab}	2.9 ± 0.4 ^{abc}	0.3
M199 + FBS + insulin	11.0 ± 1.7 ^{ab}	2.9 ± 0.6 ^{abc}	0.3
	24-h preincubated adipocytes (nmol/2 h/10^5 cells)		
KRB	3.2 ± 0.3 ^{c}	1.1 ± 0.2 ^{cd}	0.3
KRB + insulin	2.6 ± 0.3 ^{c}	1.0 ± 0.1 ^{cd}	0.4
KRB + FBS	3.8 ± 0.3 ^{c}	2.4 ± 0.2 ^{bcd}	0.6
M199	2.5 ± 0.1 ^{c}	1.1 ± 0.1 ^{cd}	0.4
M199 + insulin	2.3 ± 0.4 ^{c}	1.2 ± 0.1 ^{cd}	0.5
M199 + FBS	10.9 ± 1.7 ^{ab}	3.4 ± 0.8 ^{ab}	0.3
M199 + FBS + insulin	9.8 ± 1.0 ^{ab}	3.3 ± 1.2 ^{ab}	0.3
	****	****	

* Experiment 1; medium for preincubation was M199 without FBS; ** ratio of incorporation rate of acetate (A) to the rate of glucose (G); *** both time and medium means differ significantly ($P < 0.05$); **** the interaction between time and medium is significant ($P < 0.05$). $^{a, b, c, d, e}$ Values within a column without the same superscripts differ significantly ($P < 0.05$).

ever the incubation medium (table II). The oxidation rates of acetate to CO_2 were much lower than the rates of glucose to CO_2. The addition of FBS to M199 tended to decrease the ratio, while the addition of FBS to KRB tended to increase the ratio in preincubated adipocytes.

Comparison of different basal media on the lipogenesis and oxidation rates of acetate and glucose in fresh and preincubated isolated adipocytes

As metabolic media for the incorporation of glucose or acetate into lipid molecules and subsequent oxidation to CO_2 in isolated adipocytes during a 2-h incubation, KRB and M199 had generally the same effect (tables I and II).

Experiment 2

Cellularity

The diameter and cell number frequency distributions of adipocytes from subcutaneous adipose tissue of steers are shown in figure 1b. Although the distribution rate of large adipocytes decreased slightly while the rate of small adipocytes increased slightly, there was no significant difference in the diameter and cell number frequency distributions among fresh, 24-h preincu-

bated and 48-h preincubated adipocytes in M199 with added FBS.

The changes in lipogenesis and oxidation of acetate and glucose in isolated adipocytes preincubated in M199 with added FBS

Compared with the fresh adipocytes, adipocytes preincubated for 24 and 48 h did not show significant changes in either the incorporation rate of glucose into lipid molecules or the oxidation rates of glucose or acetate to CO_2 (table III). However, the incorporation rate of acetate into lipid molecules decreased significantly from 1117 to 711 and 307 nmol, when the incubation time extended from 0 to 24 and then 48 h, respectively.

Relative contributions of acetate and glucose to the lipogenesis and oxidation rate in isolated adipocytes preincubated in M199 with added FBS

When the culture time was extended from 0 to 24 and 48 h, the ratios of rate of incorporation into lipid molecules of acetate to glucose, decreased from 37 to 24 and 11, respectively (table III). This can be largely explained by a decrease in the incorporation rate of acetate into lipid molecules in the cells during the time of culture. The ratios of the rate of oxidation to CO_2, of acetate to glucose, remained at 0.3, whatever the culture times.

DISCUSSION

The isolating procedure removes most other cell types and leaves the isolated adipocytes, and is therefore one of the preparation methods for studying adipocyte metabolism in vitro (Hausman and Martin, 1987). In comparison with the simplicity and rapidity of tissue explant preparation, the isolation of adipocytes needs the additional time for collagenase incubation and cell separation which may result in possible damage to the cells. Based on the modifications of the Rodbell method (Yang and Baldwin, 1972; Vasilatos et al, 1983; Vernon et al, 1985), we used M199 supplemented HEPES and 4% BSA as basal medium for the collagenase digestion and for further preincubation, and tried to minimize cell breakage and possible damage to the cells. There was little difference in the diameters and cell number frequency distributions between adipocytes isolated in this method and those liberated using urea after osmium tetroxide-fixation (He et al, unpublished). On the other hand, the fragility of the cells during a prolonged

Table III. Effects of preliminary culture on the incorporation and oxidation of glucose or acetate in isolated adipocytes from four fattening steers (mean ± se)*.

	Glucose		*Acetate*		*Ratio (A/G)***	
Time	*Lipid*	*CO_2*	*Lipid*	*CO_2*	*Lipid*	*CO_2*
h	nmol/2 h/10^5 cells					
0	30.0 ± 2.2	11.2 ± 0.5	1117 ± 90 [a]	2.8 ± 0.2	37	0.3
24	25.4 ± 4.8	11.8 ± 1.7	711 ± 25 [b]	3.8 ± 0.8	28	0.3
48	27.8 ± 2.1	7.4 ± 0.8	307 ± 52 [c]	2.5 ± 2.0	11	0.3

* Experiment 2; medium for preincubation and incubation was M199 without FBS; ** ratio of incorporation or oxidation rate of acetate (A) to the rate of glucose (G); [a, b, c] Superscripts indicate that time means differ significantly ($P < 0.05$).

incubation, especially in the larger adipocytes from fattening steers, is also a possible limitation. Because of the buoyancy of isolated adipocytes, the most common method for their preliminary culture is to incubate the adipocytes under or between coverslips. Smaller adipocytes will float and may attach to the bottom of the coverslip, while large cells are presumably held between the coverslip and the lower surface. In the present study, considering the fragility of these adipocytes and to avoid the possible rupture due to the weight of the coverslip, we used standard cell culture technique and plated the isolated adipocytes with enough medium so that even floating cells would presumably be in contact with the culture substrate. Although this technique may appear less satisfying than the coverslip method, it remains to control if there is actually a significant difference. The cell distribution rates used in experiments 1 and 2 were different (fig 1a, b), although they were from the same experimental animals. One possible reason is that the second and third biopsy were not taken from the same place on the animal. The other reason may be that the extent of the collagenase digestion, the FBS and/or cell ruptures were not the same between the two experiments.

Our results on the relative contributions of acetate and glucose to lipogenesis in isolated adipocytes were similar to previous results from tissue fragments (Prior, 1983; Smith and Crouse, 1984; Miller et al, 1991; Chilliard and Faulconnier, 1995; May et al, 1995) and those using isolated adipocytes (Yang and Baldwin, 1972). In ruminants, acetate makes a major contribution to lipogenesis in adipocytes.

The oxidation rates of acetate to CO_2 were generally lower than the rate of glucose to CO_2 in adipocytes under the different conditions in the present study. They were different from the results reported by Yang and Baldwin (1972), where the oxidation rates of acetate to CO_2 were much higher than the rate of glucose to CO_2 in adipocytes, tissue slices and cell homogenates, when the rate was expressed as mμmoles substrate converted per h per mg cell protein (Yang and Baldwin, 1972). The differences in age, breed of animal and diet are the possible explanations for this.

In the isolated adipocytes incubated in media supplemented with FBS for 2 h, the incorporation rate of acetate into the total lipid decreased significantly as the length of the preincubation time increased, but there was no significant change in the incorporation rate of glucose into the total lipid contents. Our results and those in adipose tissue fragments that had been cultured for 48 h or longer are similar on this point (Miller et al, 1991; Chilliard and Faulconnier, 1995; May et al, 1995). The incorporation rates of acetate into lipid molecules in fresh adipocytes incubated in media without FBS were already very low, compared with the rates in adipocytes after being preincubated for 48 h in experiment 2 and the results in adipose tissue explants on a per cell basis (Miller et al, 1991). A possible reason may be the potential damage to the cells resulting from excessive collagenase exposure.

One of our aims in the present study was to find a suitable medium for measuring the lipogenesis of isolated adipocytes in short duration incubations. KRB is a buffer that has normally been used in short-term incubations to measure the lipogenesis of adipose tissue fragments or isolated adipocytes. M199 is a medium that has been used in the primary culture of isolated adipocytes or adipose tissue explants. Yang and Baldwin (1972) also used KRB buffer together with an amino acid mixture and other supplements as a medium for measuring the lipogenesis of isolated adipocytes. Here, we used both KRB and M199 as a short-term incubation medium to measure the incorporation rates of substrates into lipid molecules in isolated adipocytes. There was no significant differences among the incor-

poration rates of both acetate and glucose in the adipocytes incubated in the media without FBS, but the incorporation rate of glucose in preincubated adipocytes incubated in KRB + FBS was significantly higher than the rate in M199 + FBS, whereas the incorporation rate of acetate in adipocytes incubated in M199 + FBS was significantly higher than the rate in KRB + FBS, especially in the cells that were preincubated for 24 h. Further studies are needed to understand the mechanisms involved in these differences.

By activating enzymes and increasing lipogenic activity, insulin plays an important role in stimulating in vitro glucose metabolism in adipose tissues from young rats (Hausman and Martin, 1987). Most studies found little or no effect of insulin on the rate of lipogenesis in adipose tissue or isolated adipocytes from sheep and cattle when incubated in vitro for 2 h (Vasilatos et al, 1983; Miller et al, 1991; Vernon, 1992), although there have been some notable exceptions (Yang and Baldwin, 1972; Etherton and Evock, 1986). However, incubation with insulin for longer than 24 h results in a large increase in the lipogenesis rate in adipose tissues of sheep and cattle (Vernon 1979, 1992, 1996; Vernon et al, 1985; Etherton and Evock, 1986; Chilliard and Faulconnier, 1995), although there was one exception (Miller et al, 1991). With sheep adipose tissue, an effect of insulin was in fact seen when tissue was preincubated with insulin for only 5 h prior to assay of lipogenesis, and when pieces of sheep adipose tissue were preincubated without hormones for 22 h, the tissue pieces showed an acute response to insulin (Vernon, 1996). However, in the present study, the addition of insulin for 2-h in vitro incubation had no effect on lipogenesis in both fresh adipocytes and adipocytes that had been preincubated for 24 h without insulin. This is in agreement with previous results from 2-h incubations.

FBS is an extremely complex mixture of many small and large biomolecules with different, physiologically balanced growth promoting and growth inhibiting activities. Among the biological fluids that have proved to be successful for culturing cells outside the body, serum has gained the most widespread acceptance. A concentration of 5–20% serum in the medium is usually needed for optimum cell growth (Freshney, 1992). In our study, the addition of 10% FBS greatly improved the incorporation rate of both acetate and glucose into the total amount of lipids in both fresh adipocytes and preincubated adipocytes. This suggests that FBS contains factors that could change the metabolic activity of bovine adipocytes. Recently, we found a stimulatory effect of FBS on lipogenesis in isolated adipocytes taken from the subcutaneous adipose tissue of male Wistar rats of 210 days of age. The addition of 10% of FBS, 10 ng/mL insulin, or 10% FBS + 10 ng/mL insulin increased significantly the lipogenic rate in rat adipocytes during 2 h incubation in KRB with BSA, M199 with BSA or M199 without BSA (He et al, unpublished). We also found a stimulatory effect of FBS on lipogenesis in isolated adipocytes taken from the subcutaneous adipose tissue of Holstein steers at 4, 9, 22, 25 months of age and in Japanese Black steers at 14 months of age (He et al, unpublished). FBS is generally thought to be important for long-term incubation, and 10% FBS increased the release of LPL activity after heparin addition to human adipocytes in vitro (Kern et al, 1985). In the study reported by Faulconnier et al (1994), FBS prevented the positive effect of insulin on LPL activity in bovine adipose tissue explants, and the addition of FBS alone to the medium increased glucose utilization but had no effect on LPL activity or acetate utilization. In the present study, the results of experiment 2 showed that the addition of FBS to preincubation medium did not counteract the decrease of the acetate incorporation rate into lipid molecules, and

may suggest that the addition of FBS is not important for preincubation. Further studies are needed to clarify why FBS shows a significant effect on glucose and acetate incorporation into lipid molecules in isolated adipocyte during short-term in vitro incubation.

In conclusion, the main findings of this study were the interactions between basal incubation media, FBS and time of preincubation, as well as the high glucose oxidation relation to that of acetate.

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