Effect of Substrate Interactions on In Vitro Fatty Acid Synthesis in Adipose Tissue of Normal and Genetically Obese Mice

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Summary. Fatty acid synthesis was measured in vitro in pieces of adipose tissue from lean and obese-hyperglycaemic (*ob/ob*) mice, using ^{14}C -glucose or ^{14}C -lactate and $^3\text{H}_2\text{O}$ to obtain absolute rates of total fatty acid synthesis. In the presence of lipoprotein-triglyceride ($2.5\ \mu\text{mol/l}$) metabolic interaction occurred which decreased glucose incorporation into fatty acids by 30% in lean mouse tissue, but not in obese mouse tissue. In the absence of added insulin, the contribution of glucose to total fatty acid synthesis was 69% in obese mouse tissue, significantly lower than the value of 87% obtained in lean mouse tissue. Insulin increased the contribution of glucose to total synthesis in both lean and obese mouse tissues, although the value in obese mouse tissue (83%) remained lower than the value in lean mouse tissue (100%). Lactate was not a major precursor for fatty acid synthesis. When both lactate (2 mmol/l) and glucose (15 mmol/l) were present, the contribution of lactate to total fatty acid synthesis was not increased in obese mouse tissue, suggesting that even in the presence of insulin, about 30% of the carbon was provided by intracellular precursors.

Key words: Obese-hyperglycaemic, obesity, genetic, mice, fatty acid synthesis, ^3H discrimination, substrates, lactate, glucose, *ob/ob*.

Genetically obese (*ob/ob*) mice exhibit hyperinsulinaemia and hyperglycaemia, providing a model for metabolism in insulin-resistant diabetes. In these mice, increased hepatic lipogenesis has been consistently demonstrated both *in vivo* [1, 2, 3] and *in vitro* [4, 5]. Measurement of fatty acid synthesis in adipose tissue has produced conflicting results. *In vivo* studies have indicated enhanced lipogenesis in the adipose organ [2, 3, 6]. However, studies *in vitro* show increased synthesis in obese mouse tissue

measured by acetate incorporation [7], but an impairment in glucose uptake and insulin-dependent conversion of glucose to fatty acids [8].

High levels of unesterified fatty acid decrease glucose incorporation into fatty acids in adipose tissue [9]. In the obese mouse increased hepatic lipogenesis, increased triglyceride turnover [10], and increased lipoprotein lipase activity in adipose tissue [11], suggest an increased uptake of triglyceride-fatty acid by adipose tissue. We have examined the possibility that increased triglyceride-fatty acid uptake by adipose tissue of obese mice may be a mechanism by which glucose incorporation into fatty acids can be modified, by measuring the effect of lipoprotein-triglyceride (substrate for lipoprotein lipase) on glucose incorporation into fatty acids in adipose tissue *in vitro*.

It is possible that in the insulin-resistant state metabolites other than glucose may become more important substrates for fatty acid synthesis. An alternative substrate is lactate, which is a major precursor for fatty acid synthesis in rat adipose tissue [12]. To examine this possibility, ^{14}C -glucose or ^{14}C -lactate incorporation into fatty acids was measured *in vitro* when both substrates were present at concentrations approximating those *in vivo*. The discrimination against ^3H compared to ^1H also was measured, in mouse adipose tissue. This allowed calculations of absolute rates of total fatty acid synthesis from $^3\text{H}_2\text{O}$ incorporation, independent of carbon precursors, in adipose tissue from lean mice and obese mice.

Materials and Methods

Animals

Female mice were obtained from a random-bred closed colony maintained at Imperial College into which the *ob* gene was incorporated. Lean mice were either heterozygous or homozygous.

Mice were fed *ad libitum* on a standard breeding diet (Oxoid Ltd., London). Mice were studied at 5–6 weeks of age, when lean mice weighed approximately 22 g and obese mice 34 g. Female Sprague-Dawley rats were bred and maintained at Imperial College.

Chemicals

Crystalline ox insulin, glucagon-free, was prepared and supplied by Burroughs Wellcome Laboratories, Dartford, Kent. Intralipid, a 10% (w/v) emulsion of soybean oil was obtained from Vestric Ltd., Bristol. Bovine serum albumin (fraction V) was supplied by Miles Laboratories, Stoke Poges, Bucks. Radioactive isotopes were obtained from the Radiochemical Centre, Amersham. All solvents were of analytical grade; these and other chemicals were supplied by May and Baker, Dagenham, Essex.

Tissue Incubations

White parametrial adipose tissue was taken from non-fasting female mice killed by cervical dislocation between 1000 h and 1400 h. The region of brown-coloured tissue adjacent to the major blood vessels was removed since its rate of lipogenesis differed from the remaining tissue. Pieces of tissue weighing 20–50 mg were placed in gassed Krebs Ringer bicarbonate buffer (pH 7.4) [13] for a maximum of 15 min before being blotted, weighed and placed in the incubation medium.

Conditions of incubation were used which gave maximum rates of synthesis. This was dependent on the ratio tissue weight/volume of incubation medium, the size of tissue pieces, and the presence of albumin. Tissue weighing 20–50 mg was incubated at 37°C for 1 hour in sealed flasks with 2 ml Krebs Ringer bicarbonate buffer containing 2 g/100 ml defatted bovine serum albumin [14], and glucose, lactate, serum and lipoprotein-triglyceride as required. Insulin when present, had a final concentration of 25 mU/ml, which was found by previous experiments to produce maximal stimulation of lipogenesis in obese mouse adipose tissue. Similar values were required to stimulate oxidation maximally in this tissue [15]. $^3\text{H}_2\text{O}$ was present at a final specific activity of 250–500 $\mu\text{Ci/ml}$, with $\text{U-}^{14}\text{C}$ -glucose at 0.1–0.25 $\mu\text{Ci/ml}$ or $\text{U-}^{14}\text{C}$ -lactate at 0.05 $\mu\text{Ci/ml}$. Lipoprotein-triglyceride was prepared by preincubation of dialysed mouse serum and Intralipid emulsion as previously described [16]. The concentration of Intralipid-triglyceride in the incubation medium was 2.5 $\mu\text{mol/ml}$. Serum was present at 10% of its circulating concentration, and would contribute many other compounds. It was present in the same concentration in control flasks. After incubation of the tissue for 1 h, it was removed from the incubation medium with forceps and rinsed in ice-cold water before extraction of fatty acids.

Fatty acids were isolated by alkaline hydrolysis of the washed lipid extract obtained by the method of Folch et al. [17], or by direct saponification of the tissue in alcoholic KOH (40 g/100 ml 40% ethanol). Direct saponification of adipose tissue was compared with lipid extraction of the same tissue by the method of Folch et al. [17] followed by hydrolysis. 104% of the radioactivity in fatty acids (either ^{14}C or ^3H) was recovered by direct hydrolysis of the tissue compared to that recovered by lipid extraction followed by hydrolysis. Simultaneous liquid scintillation counting of the ^3H and ^{14}C content of lipids was performed using a channels ratio method [18].

The rate of fatty acid synthesis in tissues was calculated from the quotient: dpm in tissue fatty acid/dpm per μg in precursor. This value for μg atoms incorporated was converted into $\mu\text{mol-acetyl}$ units by dividing by 2 for ^{14}C . If there were no discrimination against ^3H , total synthesis in $\mu\text{mol-acetyl}$ units would be obtained

by dividing by 4 the quotient for ^3H . However, since the discrimination against ^3H (compared to ^1H) has a value of 2.94 (see Results), to obtain total synthesis in $\mu\text{mol-acetyl}$ units, the quotient for ^3H was divided by $4/2.94$ that is by 1.36.

Values are expressed as mean $\pm$ SEM. The significance of difference was analysed by Student's *t*-test.

Table 1. Fatty acid synthesis in mouse and rat parametrial adipose tissue *in vitro*. Tissue from mice (aged 5 wk) and rats (weighing 180 g) was incubated in the presence of 15 mmol/l glucose and insulin (25 mU/ml). Results are the mean $\pm$ SEM

Animal	$\mu\text{g-atoms incorporated into fatty acids/h/g fresh tissue}$			observations
	^{14}C	^3H	$^3\text{H}/^{14}\text{C}$	
Rat	43 $\pm$ 4*	35 $\pm$ 3*	0.80 $\pm$ 0.03*	8
Mouse	117 $\pm$ 21*	78 $\pm$ 13*	0.68 $\pm$ 0.02*	8

* Values significantly different, $P < 0.001$

Results

Discrimination against ^3H

Adipose tissue from mice and rats was incubated in the presence of insulin, with glucose as the only substrate. In mouse adipose tissue the $^3\text{H}:^{14}\text{C}$ ratio of μg atoms incorporated into fatty acid was 0.68 (Table 1). Assuming there was no discrimination against ^{14}C compared to ^{12}C , this gave a value of 2.94 for the discrimination against ^3H compared to ^1H , since the ratio H:C in fatty acids is 2:1. This was significantly different ($P < 0.001$) from the $^3\text{H}:^{14}\text{C}$ ratio of 0.80 in rat adipose tissue, which corresponds to a discrimination factor of 2.50.

Effect of Lipoprotein-Triglyceride

There was no significant difference in rates of fatty acid synthesis in adipose tissue from obese mice in the presence of lean or obese mouse serum, or in the presence of triglyceride 'activated' by serum (Table 2). However, in lean mouse adipose tissue, triglyceride incubated with serum from obese mice caused a significant decrease ($P < 0.05$) both in the incorporation of glucose into fatty acid and in the total rate of fatty acid synthesis, compared to the values obtained with serum alone.

Incorporation of Glucose and Lactate

In the absence of insulin, glucose incorporation into fatty acid when expressed per g tissue was similar in lean and obese mouse tissue (Table 3). The total rate of synthesis per g tissue was greater in obese mouse

Table 2. Effect of serum and triglyceride on fatty acid synthesis from glucose in parametrial adipose tissue from lean and obese mice (aged 5–6 wk). Synthesis was measured in the presence of 25 mmol/l glucose and insulin (25 mU/ml). Serum was present at 10% of its circulating concentration. Lipoprotein-triglyceride was prepared by preincubation of serum with Intralipid. The final concentration of Intralipid triglyceride was 2.5 μ mol/ml. Results are mean $\pm$ SEM. Serum at 10% of its circulating concentration contained: triglyceride < 0.1 μ mol/ml (lean and obese; free fatty acid < 0.03 μ mol/ml (lean and obese); insulin < 0.004 mU/ml (lean), 0.10 mU/ml (obese). These would be present in both control flasks (serum) and experimental flasks (serum + Intralipid). aa, bb, cc = Pairs of values between which there are significant differences, $P < 0.05$

Additions	Final conc. triglyceride μmol/ml	Fatty acid synthesis μmol-acetyl units/h/g tissue		Glucose C contribution (%)	Number of observations
		Total	Glucose C		
<i>Lean mouse tissue</i>					
Lean mouse serum	< 0.1	20.4 ± 3	18.8 ± 2	93 ± 9	3
Obese mouse serum	< 0.1	22.1 ± 2 ^b	22.3 ± 2 ^a	100 ± 2	3
Lean mouse serum + Intralipid	2.6	21.8 ± 3 ^c	20.7 ± 3	95 ± 7	3
Obese mouse serum + Intralipid	2.6	14.4 ± 1 ^{b,c}	15.1 ± 1 ^a	105 ± 2	3
<i>Obese mouse tissue</i>					
Lean mouse serum	< 0.1	24.5 ± 3	16.5 ± 2	73 ± 5	6
Obese mouse serum	< 0.1	26.2 ± 3	19.3 ± 2	74 ± 2	4
Lean mouse serum + Intralipid	2.6	21.6 ± 2	17.3 ± 2	80 ± 2	5
Obese mouse serum + Intralipid	2.6	23.5 ± 3	20.0 ± 2	86 ± 7	6

Table 3. Contribution of lactate and glucose to total fatty acid synthesis in parametrial adipose tissue from lean and obese mice (aged 5–6 wk). Results are the mean $\pm$ SEM. *, **, Values significantly different from those in appropriate obese mouse tissue. aa, bb, cc, dd, ee, ff = Pairs of values between which there are significant differences in the same tissue: ^{a,b,c,d} $P < 0.05$; ^{e,f} $P < 0.001$

Substrates	mmol/l	Fatty acid synthesis μmol-acetyl units/h/g tissue			Contribution to total synthesis (%)		Number of observations
		Total	Glucose C	Lactate C	Glucose C	Lactate C	
<i>Lean mice: no insulin</i>							
glucose	15	13 ± 3**	12 ± 3	—	87 ± 4 ^e	—	6
lactate	2	3 ± 1**	—	2 ± 1***a	—	46 ± 5*	4
glucose	15	22 ± 4	12 ± 2	6 ± 2 ^a	52 ± 3* ^c	26 ± 4	7
+ lactate	2						
<i>Obese mice: no insulin</i>							
glucose	15	20 ± 2	14 ± 2	—	69 ± 8 ^f	—	6
lactate	2	21 ± 3	—	6 ± 1	—	27 ± 4	4
glucose	15	24 ± 3	9 ± 0	4 ± 0	39 ± 5 ^f	16 ± 5	7
+ lactate	2						
<i>Lean mice: insulin 25 mU/ml</i>							
glucose	15	38 ± 10	39 ± 11	—	102 ± 5* ^b	—	5
lactate	2	2 ± 1**	—	1 ± 0***d	—	31 ± 8	4
glucose	15	41 ± 9	33 ± 7	7 ± 3 ^d	80 ± 8* ^b	17 ± 7	7
+ lactate	2						
<i>Obese mice: insulin 25 mU/ml</i>							
glucose	15	27 ± 2	23 ± 2	—	83 ± 7 ^c	—	6
lactate	2	12 ± 2	—	2 ± 0	—	20 ± 5	4
glucose	15	31 ± 4	18 ± 4	4 ± 1	59 ± 6 ^c	13 ± 3	7
+ lactate	2						

tissue. Thus the contribution of glucose to total fatty acid synthesis was greater in lean mouse tissue than in obese mouse tissue. In the presence of insulin, glucose incorporation increased $3\times$ in lean mouse tissue, but by only 50% in obese mouse tissue. The glucose carbon contribution of $102 \pm 5\%$ confirmed the discrimination factor obtained from Table 1. However, in obese mouse tissue, there was a significant carbon deficit of about 17% of the total rate of synthesis.

When lactate was the sole precursor, the incorporation of lactate carbon into fatty acids, expressed per g tissue, was significantly greater ($P < 0.001$) in obese mouse tissue. The total rates of synthesis per g tissue were also significantly greater ($P < 0.001$), the lactate incorporation being associated with a significant carbon deficit. In obese mouse tissue the deficit was about 70–80% of the total rate of synthesis. In lean mouse tissue the value was 50–70%. Insulin did not stimulate fatty acid synthesis from lactate.

When both precursors were present, substrate interaction differed in lean and obese mouse tissues. In lean mouse tissue, glucose markedly stimulated lactate incorporation ($P < 0.05$) especially in the presence of insulin. In obese mouse tissue, there was no significant stimulation of lactate incorporation by glucose. In the presence of insulin, the glucose contribution fell significantly ($P < 0.05$) in the presence of lactate to 80%, and lactate contributed 17% to total fatty acid synthesis in lean mouse tissues. In obese mouse tissues, the glucose contribution to total synthesis also fell significantly ($P < 0.05$) in the presence of lactate to 59%, lactate contributed 13% and endogenous precursors presumably supported about 30% of total synthesis.

Discussion

Earlier measurements of fatty acid synthesis in mice using ^3H incorporation from $^3\text{H}_2\text{O}$ [3, 5] calculated absolute rates of fatty acid synthesis using a factor of 2.4 to correct for the discrimination against ^3H compared to ^1H . This factor was derived for rat liver [19]. Jungas [20] obtained a value of 2.29 for rat adipose tissue. Using Jungas' method (Table 1) we obtained a value of 2.50 for rat adipose tissue, and 2.94 for mouse adipose tissue. The discrimination factor of 2.94 was used to derive rates of total fatty acid synthesis (in μmol s acetyl units) by dividing the apparent μg atoms ^3H incorporated by $4/2.94 = 1.36$.

The greater discrimination against ^3H in mouse tissue compared to rat tissue may be due to increased contribution of the pentose phosphate pathway (PPP) to the supply of reducing equivalents for fatty acid synthesis. NADPH generated by the PPP is

quantitatively recovered in fatty acids and no exchange occurs between water and hydrogen of NADPH [21]. The greater discrimination against ^3H (and thus tritium) and mouse tissue suggests the PPP provided 70–80% of the reducing equivalents for synthesis, compared with 60% in rat tissue [20]. Confirmation of this would require an analysis of the ^3H distribution on odd- and even-numbered carbon atoms of the fatty acid chain [20].

The hypothesis that increased triglyceride-fatty acid uptake by obese mouse adipose tissue may inhibit glucose incorporation into fatty acids, resulting in decreased glucose utilization was not supported. The data suggested that such inhibition may operate in normal mice, since triglyceride at a concentration approximately 3 times that *in vivo* [10], prepared as a substrate for lipoprotein lipase, did decrease fatty acid synthesis in the presence of serum. In rat adipose tissue, a concentration of 2.0 mmol/l free fatty acid was required to inhibit fatty acid synthesis [9]. It is unlikely that the lack of inhibition by triglyceride in obese mice was due to a functional decrease in lipoprotein lipase; it is more likely that it reflected a lower intracellular concentration of fatty acid in obese mouse tissue, which has an enhanced capacity for esterification [22].

Lactate was examined as an alternative substrate for fatty acid synthesis in adipose tissue. Lactate is present in the circulation in significant concentrations [23], and it is a major precursor of fatty acids both in the mouse liver [5] and rat adipose tissue [12]. In addition, *in vitro* data suggested that obese mouse tissue utilizes lactate faster than lean mouse tissue [24]. Comparison of rates of fatty acid synthesis per g tissue does not compare the activity in adipocytes of lean and obese mice, since obese mouse adipose tissue contains fewer adipocytes of increased size. Thus rates per g tissue tend to underestimate rates of fatty acid synthesis in obese mouse tissue. However, comparison of the relative importance of the two substrates is valid. When both glucose and lactate were present at concentrations approximating those *in vivo*, it was found that unlike the liver, lactate was not a major precursor for fatty acid synthesis (supporting about 17% of the total). Moreover, lactate did not become a more important precursor in the insulin-resistant adipose tissue of the obese mouse.

Lactate utilization appeared to be associated with a large contribution (50–80%) by intracellular precursors to total fatty acid synthesis. It is possible that this is an overestimate. When no glucose is present, the activity of the PPP would be minimal and the discrimination factor of 2.94 would be too high. Total synthesis may be overestimated by up to 75%. However, lactate carbon incorporation would still be insufficient to account for total synthesis. In the

presence of lactate, intracellular precursors would support a minimum of 25–65% of total fatty acid synthesis.

A greater proportion of fatty acid synthesis also appeared to be maintained by intracellular precursors in obese mouse adipose tissue compared to lean mouse tissue, as suggested by Christophe et al. [25]. It is unlikely that this is an artefact caused by the discrimination factor being too high, due to a decreased role of the PPP in obese mouse adipose tissue [26]. Enzyme activity measurements indicated an elevated role for the PPP, since the activity of 6-P-gluconate dehydrogenase was increased, whereas the activity of malic enzyme was not [27].

It is possible that a major intracellular precursor of fatty acids was glycogen. Glycogen facilitates lactate utilization [21], and the increased utilization of lactate (in the absence of glucose) and the greater carbon deficit in obese tissue may reflect the higher concentration of glycogen [28]. The glycogen concentration of about 1 μmol glycogen-glucose/g reported by these authors would be insufficient to support the rates of synthesis observed in obese mice, suggesting other metabolites such as amino-acids may play a significant role as fatty precursors in obese mouse adipose tissue.

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