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Effect of insulin on human adipose tissue metabolism in situ. Interactions with beta-adrenoceptors

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Summary. The effects of insulin, and its interactions with catecholamines through beta-adrenoceptors, on human adipose tissue glucose utilization and lipolysis were investigated in vivo. Microdialysis of the extracellular compartment of abdominal subcutaneous adipose tissue was performed in healthy subjects of normal weight, before and during a 2-h hyperinsulinaemic (61 ± 3 mU/l), euglycaemic clamp. The tissue was perfused with or without the beta-adrenergic agonist isoproterenol (10^{-6} mol/l), and the tissue dialysate concentrations of glucose, glycerol (lipolysis index) lactate and pyruvate were determined. During the insulin infusion, glucose in adipose tissue decreased by 20 % ($p < 0.001$), despite arterial steady-state normoglycaemia. The concentrations of lactate and pyruvate increased gradually to a steady-state plateau of twice the basal level in adipose tissue and arterial blood. Insulin-induced suppression of glycerol (lipolysis index) was, if anything, more marked in adipose tissue than in plasma (65 % vs 50 % decrease from baseline levels, $p < 0.05$). In situ perfusion of adipose tissue with isoproterenol, starting either at the beginning of the study period or at 45 min after initiation of the insulin infusion, resulted in marked and rapid elevations of all the investigated metabolites in the adipose tissue extracellular compartment ($p < 0.05$–0.005). It is concluded that insulin action on glucose uptake and lipolysis in human adipose tissue in vivo is counteracted by beta-adrenoceptor stimulation. In contrast, insulin and beta-adrenoceptors have synergistic effects on non-oxidative glucose metabolism in human adipose tissue in situ.

Key words: Microdialysis, glucose, glycerol, lactate, pyruvate, isoproterenol.

Adipose tissue metabolism plays a key role in the overall regulation of lipid and carbohydrate homeostasis in man. Firstly, non-esterified fatty acids derived from adipose tissue lipolysis constitute the major source of fuel during prolonged exercise and long-term catabolic conditions [1]. Secondly, the amount of ingested glucose taken up by adipose tissue in humans may be larger than previously thought [2]. Moreover, recent data have demonstrated that lactate formation occurs in human adipose tissue in vivo following oral glucose intake [3–5]. Since part of the post-prandial glycogen synthesis in the liver is believed to take place via an indirect pathway which is dependent on conversion of glucose to lactate and other three-carbon compounds in peripheral tissues [6–8], the latter finding may further emphasize the importance of adipose tissue glucose metabolism in the regulation of carbohydrate homeostasis.

In adult humans, insulin and catecholamines are the major hormonal regulators of adipose tissue metabolism [9, 10]. Insulin promotes the uptake and intracellular metabolism of glucose, and inhibits lipolysis. Catecholamines, acting via beta-adrenoceptors, counteract insulin action on lipolysis and glucose uptake in fat cells [11, 12]. However, it is not clear whether there are any interactions between insulin and catecholamines on the further metabolism of glucose within the fat cell.

Most of our knowledge of hormone action in fat cells is derived from in vitro or in vivo studies of circulating metabolites. Recently, the microdialysis technique has been introduced for studies of human adipose tissue metabolism in situ [13, 14]. With this technique it is possible to simultaneously monitor several substrates in the extracellular compartment in subcutaneous fat. In addition, the method makes possible the delivery of metabolically active hormones or drugs to this compartment through the microdialysis device in situ while avoiding the systemic effects of these compounds [3, 15–18], and it may therefore be used for mechanistic studies of the hormonal regulation of adipose tissue metabolism in situ [16].

In the present study we have investigated in vivo and in situ the effects of insulin and its interactions with catecholamines on human adipose tissue lipolysis and glucose

metabolism by combining the microdialysis method with the hyperinsulinaemic, euglycaemic clamp technique [19]. Microdialysis probes were implanted in the abdominal subcutaneous adipose tissue in healthy non-obese subjects. The probes were continuously perfused using solvents with or without the synthetic beta-adrenergic agonist isoproterenol. The tissue dialysate levels of glucose, glycerol (lipolysis index), lactate and pyruvate were continuously monitored.

Subjects and methods

Subjects

The study group comprised 14 healthy subjects (nine men and five women), aged 20–52 years (mean ± SEM; 36 ± 3). All subjects were of normal weight (body mass index, 22.3 ± 0.4 kg/m^2). None had taken drugs known to alter adipose tissue metabolism and none showed evidence of a recent weight change. The study was approved by the ethics committee of the Karolinska Institute. The subjects were each given a detailed description of the study and their consent was obtained.

Microdialysis probe

The probe (CMA Microdialysis AB, Stockholm, Sweden) has previously been described in detail [20]. Briefly, a dialysis tubing (10 × 0.5 mm, M_r cut-off = 20,000) is glued to the end of a double lumen catheter. The inlet tubing of the probe is connected to a high-precision microinfusion pump (CMA/100 microinjection pump; CMA Microdialysis AB) and is continuously perfused with a sterile solution. The perfusion fluid enters through the inner cannula, passes down to the tip of the probe, streams upwards in the space between the inner cannula and the dialysis membrane, and leaves the probe through the outer cannula via a side-arm from which it is collected.

The characteristics of the microdialysis probe in vitro and in subcutaneous adipose tissue in vivo have previously been presented in detail [3, 15, 16, 21]. Briefly, at a given perfusion speed the relative recovery in vitro (dialysate substrate concentration vs medium substrate concentration × 100) of glucose, glycerol, lactate and pyruvate remains constant over a wide range of substrate concentrations. The concentrations of these substrates in the adipose tissue dialysate remain the same for at least 2 h during steady-state conditions. Moreover, the in vivo metabolite recovery is identical whether or not the investigated substance is added to the perfusion medium. The latter findings indicate that there is neither drainage of the substrates from the extracellular compartment to the tissue dialysate nor oedema or hyperaemia around the dialysis membrane that interferes with dialysis recovery. Moreover, since enzymes and other large molecular weight substances are too large to pass through the dialysis membrane, the tissue samples are protected from enzymatic degradation [22].

Study protocol

The subjects were investigated in the supine position at 08.00 hours, after an overnight fast. The hyperinsulinaemic, euglycaemic clamp was performed by DeFronzo's method [19]. A catheter for infusates was inserted, following local anaesthesia, into the axillary vein via an antecubital vein. A second catheter was placed in a dorsal vein of the ipsilateral hand for blood sampling. The hand was kept in a warm box (60°–70°C) to provide arterialization of venous blood [23]. A priming plus continuous infusion of crystalline human insulin (Actrapid Human; Novo A/S, Bagsvaerd, Denmark), 1 mU · kg^{-1} · min^{-1}, was given for 2 h. Euglycaemia (the fasting glucose concentration ± 10 mg/100 ml) was maintained by a variable 20% glucose infusion. The infusion rate was determined by measurements of arterialized plasma glucose (Beckman Glucose Analyzer II; Beckman Instruments, Fullerton, Calif., USA) every 5 min. Plasma concentrations of glucose [24], glycerol [25], lactate [26], pyruvate [27] and free insulin [28] were analysed in the basal state (mean value of three samples obtained at −10, −5 and 0 min before the start of the insulin infusion), and every 15 min during the 2 h insulin clamp.

A microdialysis probe was inserted percutaneously, under sterile conditions, into the subcutaneous adipose tissue in the periumbilical region, using a steel guide cannula, anaesthesia was not needed. The probe was continuously perfused (5 µl/min) with Ringer's solution (content of Ringer's solution in 1000 ml: 147 mmol sodium, 4 mmol potassium, 2.3 mmol calcium, 156 mmol chloride. Osmolality: 290 mosm/kg). Following an equilibration period of 30 min, the tissue dialysate was collected in 15-min fractions beginning with two 15-min baseline samplings before the start of the insulin clamp, and the dialysate concentrations of glucose [29], glycerol [25], lactate [22] and pyruvate [22] were determined. The coefficient of variation for the duplicate determinations of tissue dialysate concentrations in the basal state was less than 10% for all the metabolites investigated.

In seven of the subjects, two additional microdialysis probes were inserted to the same subcutaneous fat depot, the distance between each probe being about 3 cm. Immediately after implantation one probe was continuously perfused with Ringer's solution supplemented with 10^{-6} mol/l isoproterenol and 1 mg/ml ascorbic acid (to prevent oxidation of the catecholamine analogue). The other was initially perfused with Ringer's solution alone, and at 45 min after the start of the insulin infusion, isoproterenol (10^{-6} mol/l) plus ascorbic acid (1 mg/ml) were added. Ascorbic acid alone did not influence any of the measured metabolite concentrations in the adipose tissue dialysate [17, 22, 29]. The tissue dialysate samples were collected as described. Recent microdialysis experiments have shown a transient lipolytic effect of isoproterenol when human adipose tissue was perfused with low concentrations ($\leq 10^{-8}$ mol/l) of this agent [17, 30]. In methodological experiments it was observed that perfusion with 10^{-6} mol/l of isoproterenol caused a rapid and marked increase in the glycerol level of adipose tissue, which remained constantly elevated during at least 2 h of perfusion.

Statistical analysis

The reported values are the means ± SEM. The total area under the curve (AUC) was calculated by trapezoidal integration. The Student's paired *t*-test was used for statistical evaluation of the results.

Results

The fasting arterial plasma free insulin concentration was 6.2 ± 0.6 mU/l. The insulin infusion resulted in a steady-state level of insulin of 61 ± 3 mU/l, which remained unaltered throughout the experiment. The coefficient of variation for each individual insulin plateau was 6.4 ± 1.0%.

The fasting arterial plasma glucose level was 4.8 ± 0.1 mmol/l. The subjects were clamped at their individual fasting glucose levels. The coefficient of variation for the glucose levels during the second hour of the clamp was 3.7 ± 0.4% (6.2 ± 0.4% for the whole period). The total body glucose uptake (M) was calculated using data from the second hour of the clamp. During this period the glucose disposal rate was 7.4 ± 0.5 mg · kg^{-1} · min^{-1}.

While the arterial plasma glucose was maintained at almost constant levels during the hyperinsulinaemic clamp

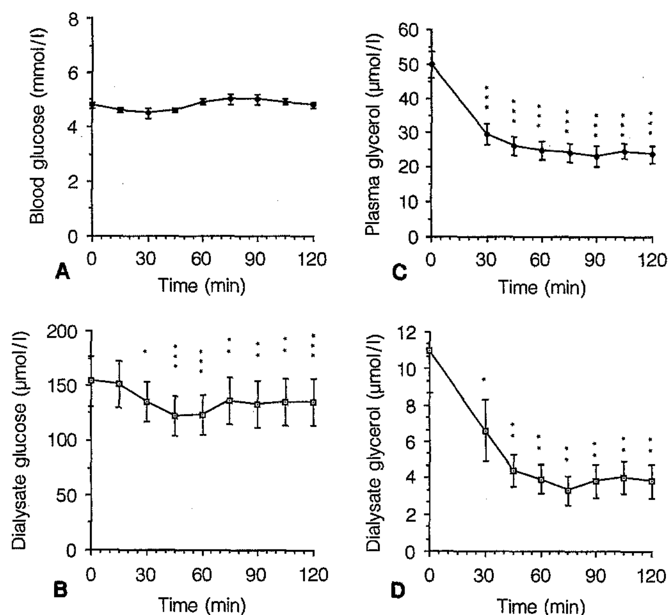

Fig. 1 A–D. Glucose and glycerol concentrations in arterial plasma (upper panel) and in adipose tissue (lower panel) during a hyperinsulinaemic, euglycaemic clamp. In 14 subjects a microdialysis probe was implanted in the abdominal subcutaneous adipose tissue and continuously perfused with Ringer's solution. After an equilibration period of 30 min, dialysate fractions of 15 min each were sampled. After two baseline samples were taken, the 2-h insulin clamp (0.1 $mU \cdot kg^{-1} \cdot min^{-1}$) was started. Values are the mean ± SEM. Concentrations at individual time-points were compared to baseline levels using the Student's *t*-test for paired differences. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

(Fig. 1 A), the glucose concentration in the adipose tissue dialysate gradually fell by about 20 % from the basal value ($p < 0.001$) during the first hour of the clamp. Thereafter it remained at this decreased level throughout the study period (Fig. 1 B).

The fasting arterial plasma glycerol concentration was 49.8 ± 3.8 µmol/l. After initiation of the insulin infusion, plasma glycerol levels rapidly decreased and were significantly suppressed by about 50 % ($p < 0.001$), as compared to the baseline value during the second hour of the clamp (Fig. 1 C). The kinetic pattern of glycerol in adipose tissue was similar to that in plasma, with a gradual fall during the first half of the clamp (Fig. 1 D). However, in relative terms, the maximum decrease in glycerol in adipose tissue (approximately 65 %) was significantly greater ($p < 0.05$) than that in arterial blood. In both compartments the lactate and pyruvate concentrations gradually increased in a similar fashion within the first hour of the clamp, to reach a stable plateau of about twice the respective baseline values ($p < 0.01$–0.001) during the second hour of hyperinsulinaemia (Fig. 2 A–D).

Effect of isoproterenol

Before the insulin infusion (i.e. under basal conditions) the dialysate concentration of glucose was 50 % higher ($p < 0.025$) in the fat tissue perfused with 10^{-6} mol/l isoproterenol than in the two probes which contained Ringer's solution alone (Fig. 3 A). During the hyperinsulinaemic clamp, tissue dialysate glucose decreased by 20 % ($p < 0.05$–0.005) whether or not isoproterenol had been added to the tissue perfusate from the beginning. When the tissue perfusate was changed from Ringer's solution to isoproterenol at 45 min after the initiation of the insulin infusion, a rapid and significant ($p < 0.05$) increase in the dialysate glucose concentration to the same level as that when the tissue was perfused with isoproterenol from the start was recorded (Fig. 3 A).

In situ perfusion with isoproterenol also markedly influenced the adipose tissue glycerol levels (Fig. 3 B). In the basal state, the dialysate concentration of glycerol in the isoproterenol probe was almost four-fold higher than in the Ringer probes ($p < 0.025$). Following systemic insulin administration, the glycerol levels decreased by only about 20 % ($p < 0.005$) when the tissue perfusate was supplemented with 10^{-6} mol/l isoproterenol, as compared to a 50 % reduction in tissue dialysate glycerol in the Ringer probes. Moreover, during hyperinsulinaemia the tissue glycerol level remained 6–7 times higher ($p < 0.05$–0.01) in the presence than in the absence of isoproterenol. Likewise, when the perfusion medium was changed from Ringer's solution to isoproterenol during the clamp, an immediate seven-fold increase ($p < 0.005$) in the dialysate glycerol concentration was recorded, which reached the same level as that in the probe that contained isoproterenol from the start of the experiment (Fig. 3 B).

The effects of in situ isoproterenol perfusion on adipose tissue lactate and pyruvate levels are depicted in Figure 3 C–D. Addition of isoproterenol to the tissue perfusate did not significantly alter the tissue dialysate basal concentrations of lactate and pyruvate. However, the

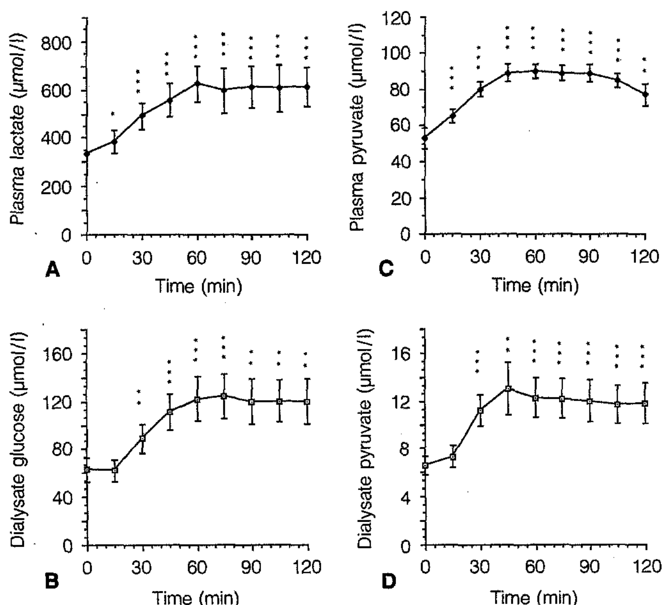

Fig. 2 A–D. Lactate and pyruvate concentrations in arterial plasma (upper panel) and in adipose tissue (lower panel) during a hyperinsulinaemic, euglycaemic clamp. See legend to Figure 1 for further details

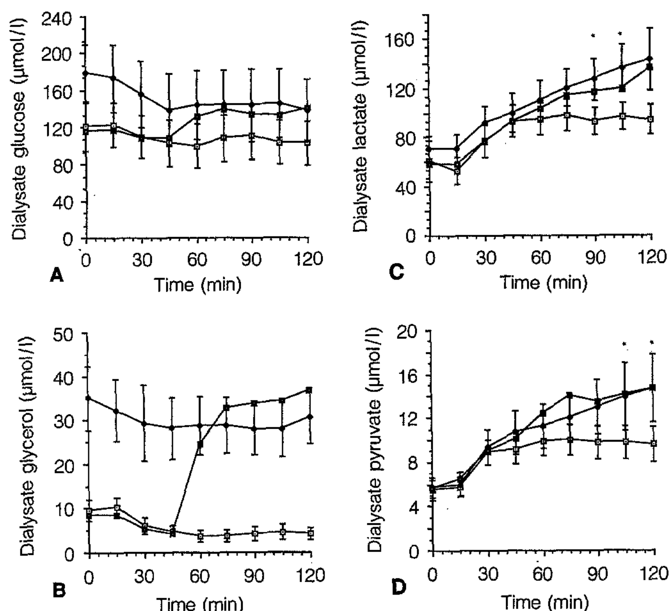

Fig. 3 A–D. Effects of isoproterenol on adipose tissue dialysate glucose (**A**), glycerol (**B**), lactate (**C**) and pyruvate (**D**) levels before and during a hyperinsulinaemic, euglycaemic clamp. Three dialysis probes were implanted in the abdominal subcutaneous adipose tissue region in seven healthy subjects. Two probes were perfused with either Ringer's solution (□) or isoproterenol (10^{-6} mol/l) (◆) from the start of the experiment. The third probe was initially perfused with Ringer's solution, but 45 min after the start of the insulin infusion the tissue perfusate was changed to isoproterenol (10^{-6} mol/l) solution (■). See legend to Figure 1 for further details

kinetic profiles of adipose tissue lactate and pyruvate during systemic hyperinsulinaemia differed markedly whether or not isoproterenol had been added to the tissue perfusate. In the absence of isoproterenol, tissue lactate and pyruvate gradually increased within the first hour and then reached steady-state levels during the second hour of the clamp. In contrast, when isoproterenol was added from the start of the experiment the tissue dialysate concentrations of the two metabolites continued to increase throughout the study period, and during the last 30–45 min of the experiment the concentrations of lactate and pyruvate in the isoproterenol probe were about 40% higher than those in the Ringer probe ($p < 0.05$, comparing individual values or area under the curve). When the in situ isoproterenol perfusion was initiated at 45 min after the start of the insulin infusion, the same pattern was observed – i. e. the dialysate concentrations of lactate and pyruvate were markedly enhanced to levels comparable to those when the tissue was perfused with isoproterenol from the beginning of the study period.

Discussion

After initiation of the insulin infusion, arterial insulin rose to a steady-state level of about 55–60 mU/l above baseline values, and arterial plasma glucose was held constant at the fasting level. By contrast, the glucose concentration in the adipose tissue dialysate decreased significantly by approximately 20%, despite steady-state glycaemia. This finding indicates that the glucose uptake in adipose tissue was markedly facilitated by insulin. This is supported by the recent finding of Coppack et al. [31], who by measuring arteriovenous differences across the abdominal wall, demonstrated a net glucose uptake in the tissue during a hyperinsulinaemic, euglycaemic glucose clamp.

While the kinetic pattern of glycerol in subcutaneous adipose tissue was similar to that in arterial blood the decrease in glycerol in the adipose tissue dialysate was, in relative terms, more marked than that in plasma. However, this difference may be more apparent than real since in the post-absorptive state the absolute glycerol concentration in the adipose tissue interstitial compartment is 2–3 times higher than the circulating glycerol level [16, 32]. Hence, these findings, which accord with recently performed in vivo microdialysis [18] and arteriovenous difference [31] studies in humans, suggest a marked and sustained inhibition of the lipolytic activity in fat cells during systemic hyperinsulinaemia.

In accordance with the results of previous reports [31, 33], we found that the plasma levels of lactate and pyruvate increased during the hyperinsulinaemic, euglycaemic clamp, indicating an enhanced rate of the overall non-oxidative glucose utilization. While several recent studies [3–5] suggest that adipose tissue in humans may be an important source of lactate production in vivo, it is not possible from the present findings to estimate the contribution of lactate derived from subcutaneous fat, since the kinetic pattern of lactate (and pyruvate) in the adipose tissue extracellular space was indistinguishable from that in arterial blood. However, the finding that perfusion of the tissue with isoproterenol markedly influenced the concentrations of these metabolites in the tissue dialysate together with results from arteriovenous difference measurements [31], suggest that in situ adipose tissue lactate and pyruvate production occur in response to insulin stimulation.

The new findings in this study relate to the interactions between insulin and catecholamines. Here we used a pure synthetic beta-adrenergic agonist, isoproterenol, because it is well known that catecholamines, via beta-adrenoceptor stimulation, reduce insulin-induced glucose transport activity and counteract the antilipolytic action of insulin in fat cells [11, 12, 34]. Moreover, recent microdialysis studies [17, 30], have shown that continuous in situ perfusion of human adipose tissue with low concentrations of isoproterenol ($\leq 10^{-8}$ mol/l) have only a transient and varying lipolytic effect. This was probably due to the rapid desensitization of beta-adrenoceptors. In contrast, local exposure of the tissue to the presently-used high concentration of isoproterenol (10^{-6} mol/l) caused a rapid and sustained increase in glycerol in the adipose tissue dialysate, which indicates that the tachyphylaxia phenomenon may be overcome by high concentrations of the non-selective, beta-adrenergic agonist. Hence, it was necessary to use the high isoproterenol dose to ensure prolonged and reproducible beta-adrenoceptor activation when the interactions between insulin and beta-adrenergic stimulation were investigated. In the present experiments, in situ perfusion with isoproterenol in the basal state significant-

ly raised the glucose concentration in the tissue dialysate and when the tissue was exposed to isoproterenol in the middle of the insulin infusion, a similar increase in adipose tissue glucose was recorded. This increase in glucose in the extracellular water space may have been the result of a beta-adrenoceptor-mediated inhibition of adipocyte glucose uptake induced by basal and elevated systemic insulin concentrations. However, during submaximally effective hyperinsulinaemia [35], the relative decrease in interstitial glucose was comparable, whether or not isoproterenol was added to the dialysis solvent from the start of the experiment. Taken together, these findings indicate that maximum beta-adrenoceptor stimulation cannot completely counteract insulin-induced human adipose tissue glucose uptake at physiological insulin concentrations.

Concerning the interplay between insulin and catecholamines on the in situ adipose tissue lipolytic activity, the stimulatory influence of isoproterenol seemed to almost completely override the antilipolytic effect of insulin. In the basal state the tissue glycerol level was markedly elevated in the presence of isoproterenol, and tissue glycerol increased to the latter level when isoproterenol was added after insulin during the hyperinsulinaemic clamp. Moreover, the lipolysis rate was markedly less inhibited by insulin in the presence than in the absence of isoproterenol. The circulating concentration of insulin during the clamp was probably maximally effective as regards in vivo antilipolysis [36]. With respect to human fat-cell lipolysis in vitro it has recently been shown that a maximally effective beta-adrenoceptor stimulation almost completely counteracts a maximal insulin-receptor stimulation [37]. The present data suggest that the same is true of human adipose tissue lipolysis regulation in situ.

Isoproterenol also markedly influenced the adipose tissue lactate and pyruvate levels during systemic hyperinsulinaemia with a continuous increase of both metabolites throughout the 2-h study period. A similar further increase in the adipose tissue lactate/pyruvate levels was also recorded when isoproterenol was added to the dialysis solvent in the middle of the study period. These findings strongly indicate a synergistic stimulation of lactate/pyruvate production (i.e., non-oxidative glucose utilization) in human adipose tissue in vivo by the combined stimulation of insulin receptors and beta-adrenoceptors. They were unexpected since we have previously shown, using microdialysis, that isoproterenol reduces the adipose tissue lactate formation in situ during an oral glucose load [3]. However, since the amount of glucose metabolized non-oxidatively is influenced not only by insulin but also by the rate of glucose uptake and the circulating glucose concentrations [38, 39], the present and previous [3] experiments may not be comparable. Hence, from the current results it appears that under euglycaemic conditions, the intracellular glucose utilization in human fat cells is routed towards non-oxidative pathways and an enhanced lactate/pyruvate production during combined insulin-catecholamine stimulation.

It should be stressed that microdialysis measurements reflect the net sum of events at the fat-cell and vascular levels. Thus, it might be argued that the observed changes in adipose tissue metabolite levels were influenced by alterations in the adipose tissue blood flow (ATBF) as well as in the adipocyte metabolism. In theory, both insulin and isoproterenol may increase the local blood flow [40, 41]. It was not possible to evaluate this parameter by means of conventional techniques (i.e. the ^{133}Xe washout technique; [42]) because of the diminutive radius of the tissue around the microdialysis probe in which the perfused substance will spread [43, 44]. However, taken together the conclusions regarding the data with glycerol, lactate and pyruvate would be the same, or even more definite, when a possible increase in ATBF is considered. Since these metabolites are delivered from the fat cells, an increase in ATBF should lead to the depletion rather than the accumulation of the substances because of the enhanced removal by the microcirculation. The glucose levels in the tissue dialysate, on the other hand, were probably influenced by vascular factors. In situ perfusion with isoproterenol, whether instituted from the start or the middle of the study period, significantly raised the dialysate glucose concentration by 50%. This finding would appear at odds with the general concept of a rapid equilibration of glucose between the arterial circulation and the interstitial tissue compartment. Hence, the increase in dialysate glucose, may be at least partly explained by an enhanced blood flow induced by the beta-adrenergic agonist, leading to a non-linear relationship between the extracellular and the dialysate glucose concentrations. Support for this notion was given in a recent microdialysis study [45] where an increase in skeletal muscle blood flow was accompanied by a simultaneous increase in the tissue dialysate glucose concentration.

Note also that the absolute metabolite concentrations in the adipose tissue interstitial compartment were not determined in this study. The finding that the dialysate concentrations of metabolites were lower than those in plasma is due to incomplete recovery in the microdialysis experiments. It is possible, using a calibration technique, to indirectly estimate the true tissue metabolite concentrations with microdialysis during steady-state conditions [46]. It was not possible to include this method in the present experiments. The calibration experiments are very time-consuming and necessitate local exposure of the tissue to very high concentrations of the substance being investigated, which may artifically influence the result of a subsequent kinetic experiment. However, the tissue dialysate concentrations of the metabolites were almost the same in the two microdialysis probes that were perfused with Ringer's solution alone from the start of the experiments. In conjunction with previous methodological data [16], this demonstrates the high reproducibility of the microdialysis technique.

In conclusion, the results of this microdialysis study have shown that insulin receptor stimulation and beta-adrenoceptor stimulation have both antagonistic and synergistic effects on human adipose tissue metabolism in situ. Insulin stimulates glucose uptake and inhibits the lipolysis rate. These effects are counteracted by beta-adrenergic stimulation. In addition, a synergistic stimulatory influence of the two hormone receptor systems on

adipose tissue lactate and pyruvate production can apparently be seen in vivo.

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