Proceedings of the Nutrition Society (2017), **76**, 419–424 doi:10.1017/S0029665116003013
 First published online 19 January 2017

Nutrition Society Summer Meeting 2016 held at University College Dublin 11–14 on July 2016

Conference on 'New technology in nutrition research and practice' Symposium 5: Advanced phenotyping in nutrition research

Characterisation of fatty acid metabolism in different insulin-resistant phenotypes by means of stable isotopes

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The obese insulin resistant and/or prediabetic state is characterised by systemic lipid overflow, mainly driven by an impaired lipid buffering capacity of adipose tissue, and an impaired capacity of skeletal muscle to increase fat oxidation upon increased supply. This leads to the accumulation of bioactive lipid metabolites in skeletal muscle interfering with insulin sensitivity via various mechanisms. In this review, the contribution of dietary *v.* endogenous fatty acids to lipid overflow, their extraction or uptake by skeletal muscle as well as the fractional synthetic rate, content and composition of the muscle lipid pools is discussed in relation to the development or presence of insulin resistance and/or an impaired glucose metabolism. These parameters are studied *in vivo* in man by combining a dual stable isotope methodology with [$^2H_2$]- and [U-^{13}C]-palmitate tracers with the arterio-venous balance technique across forearm muscle and biochemical analyses in muscle biopsies. The insulin-resistant state is characterised by an elevated muscle TAG extraction, despite similar supply, and a reduced skeletal muscle lipid turnover, in particular after intake of a high fat, SFA fat meal, but not after a high fat, PUFA meal. Data are placed in the context of current literature, and underlying mechanisms and implications for long-term nutritional interventions are discussed.

Fatty acids: Insulin resistance: Skeletal muscle: Dual stable isotope methodology: Dietary fat quality

The prevalence of overweight and overweight-related metabolic disturbances is increasing at an alarming rate. Worldwide more than 50 % of the adults is overweight (>one billion individuals) and a further 12 % (475 million) can be classified as clinically obese. Every year at least 2·8 million adults die as a result of being overweight/obese (http://www.who.int/gho/ncd/risk_factors/overweight/en). Obesity is an important risk factor for chronic metabolic diseases such as type 2 diabetes mellitus (T2DM) and CVD.

A disturbed lipid metabolism in multiple tissues, including adipose tissue, liver, skeletal muscle, gut and pancreas may play an important role in the development of insulin resistance (IR), an impaired glucose metabolism and T2DM. These disturbances, in particular an impaired adipose tissue lipid handling, may lead to systemic lipid overflow, increased circulating concentrations of NEFA and TAG and accumulation of lipids in non-adipose tissues[1–4]. This lipid overflow together with an impaired capacity to adjust fatty acid (FA) oxidation to FA supply in skeletal muscle (metabolic inflexibility[3]) may cause excess fat storage in skeletal muscle, which is related to the development or worsening of IR. IR in concert with progressive β-cell failure leads to an increased blood glucose concentration in the non-diabetic range, classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). IFG (fasting glucose >5·6 mM/l) and IGT (2 h oral glucose tolerance test-derived glucose concentration >7·8 mM) are intermediate states in the transition from a normal glucose tolerance towards T2DM. IFG and IGT may represent distinct pathways towards T2DM, with impaired hepatic and peripheral insulin sensitivity as the predominant disorders in IFG and IGT subjects, respectively[5–7]. Recently, a dual-stable-isotope tracer approach was validated to study FA partitioning, the metabolic fate of dietary compared with

Abbreviations: DAG, diacylglycerol; E%, % of energy expenditure; FA, fatty acid; FSR, fractional synthetic rate; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IR, insulin resistance; LPL, lipoprotein lipase; T2DM, type 2 diabetes mellitus.

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endogenous FA and skeletal muscle FA metabolism in detail[8]. This methodology was used to describe more into detail FA partitioning and skeletal muscle FA handling in the overweight insulin-resistant and insulin-sensitive states and in IFG and IGT subjects.

Dual-stable-isotope methodology

As indicated earlier, a combination of differential stable isotope labelling of endogenous and meal-derived FA, with arteriovenous tracer and tracee concentration difference measurements across forearm muscle was used to study endogenous FA and skeletal muscle FA metabolism in detail[8]. The aim was to quantify the systemic concentration and forearm muscle uptake of FA, derived from chylomicron–TAG (labelled with [U-^{13}C]-palmitate), VLDL–TAG (labelled endogenously with [$^{2}H_2$]-palmitic acid) and circulating NEFA (labelled with [$^{2}H_2$]-palmitic acid). On a study day, repeated blood samples were taken from the radial artery (or from a arterialised dorsal hand vein) and from the deep anticubital forearm vein (canula was placed in retrograde direction) during overnight-fasted conditions and after the ingestion of a high-fat mixed meal (2·6 MJ, 61 % of energy expenditure (E%) fat (35·5 E % saturated fat)), containing 200 mg [U-^{13}C]-palmitate (98 % enrichment, Cambridge Isotope Laboratories, Andover MA) to label chylomicron–TAG. Subjects also received a continuous intravenous infusion of [$^{2}H_2$]-palmitate (97 % enrichment, Cambridge Isotope Laboratories), complexed to human albumin, which commenced 60 min before the blood sampling (0·035 μM/kg body weight per min). In parallel, skeletal muscle biopsies (m. vastus lateralis) were taken before the start of the experiment during fasting and at the end of the postprandial measurement period to determine intramuscular TAG, diacylglycerol (DAG), NEFA and phospholipid content, their degree of saturation as well the fractional synthetic rate (FSR) of TAG, DAG and phospholipid[8–12].

The insulin-resistant state is associated with an altered muscle lipid handling

As indicated earlier, systemic lipid overflow, which may be related to adipose tissue dysfunction and disturbances in hepatic and skeletal muscle lipid handling is associated with IR[4]. This may result in an increased supply of FA to non-adipose tissues, such as liver and skeletal muscle. Due to an impaired capacity to increase fat oxidation accordingly (metabolic inflexibility[3]), TAG may accumulate, which is associated with skeletal muscle IR and which is already present in young lean offspring of T2DM subjects[13]. In line, both high-fat diets and acute intra-lipid infusions resulted in an increased skeletal muscle TAG accumulation and a concomitant development of muscle insulin resistance[4,14,15]. Nevertheless, several studies reported a similar muscle TAG content in obese insulin-sensitive subjects and obese T2DM subjects[16,17], whilst highly insulin-sensitive athletes exhibited high muscle TAG concentrations[17,18]. These findings may be explained by a higher muscle oxidative capacity in athletes, whilst the obese insulin-resistant or diabetic state is often characterised by a reduced muscle oxidative capacity[4]. During recent years, insight has increased that a complex interplay between FA supply, fat quality, muscle lipid turnover and the subcellular composition and localisation of bioactive lipid metabolites, such as DAG, long-chain fatty acyl-CoA and ceramides, is involved in the development of skeletal muscle IR (for review see[4]).

Skeletal muscle lipid uptake

Not much is known on the contribution of dietary FA (chylomicron–TAG) and endogenous FA (NEFA and VLDL–TAG) to lipid overflow and skeletal muscle FA handling. There is controversial evidence for the notion that an increased adiposity is related to enhanced fasting, postprandial, diurnal or nocturnal NEFA concentrations[19]. Elevated TAG concentrations may be more closely associated with the insulin-resistant state, which may be ascribed to an increased hepatic VLDL–TAG production[20] or a reduced adipose tissue TAG clearance from the circulation[21,22]. Furthermore, reduced suppression of FA spillover from TAG-derived hydrolysis across adipose tissue has been shown in obese patients with T2DM compared with healthy controls[21] and in insulin resistant as compared with insulin-sensitive subjects[23]. An increase in adipose tissue NEFA output may lead to an increased hepatic VLDL production[23]. It was demonstrated that dietary FA were preferentially taken up in skeletal muscle and adipose tissue in healthy volunteers despite the presence of a higher percentage VLDL–TAG in the circulation[20]. So far, most studies focusing on the contribution of chylomicron–TAG and endogenous FA to systemic lipid overflow and skeletal muscle lipid handling have been performed in healthy lean human subjects[8,24]. Up to now, elevated VLDL–TAG concentrations have been reported in overweight men and women with IR[20,23]. Additionally, in an earlier study we have shown that overweight insulin-resistant men with the metabolic syndrome show an increased forearm muscle VLDL–TAG extraction[9].

Recent studies compared fasting and postprandial skeletal muscle FA handling, firstly, in individuals with varying degree of IR[10], and, secondly, in pre-diabetic subjects with IFG and/or IGT[11]. In the first study, seventy-four overweight participants (males and females) were divided in two groups based on the homeostasis model assessment for IR median. In the second study, twelve subjects (males and females) with IFG and fourteen subjects with IGT (or combined IFG/IGT) were studied. In the latter study, postprandial insulin sensitivity was reduced and peripheral insulin sensitivity tended to be reduced in IGT as compared with IFG subjects. This is in line with previous studies showing impaired hepatic and peripheral IR as primary disorders in IFG and IGT subjects, respectively[5–7], indicating that the development of IR may be tissue-specific and that IFG and/or IGT may represent distinct pathways towards T2DM.

In the two studies, fasting and postprandial skeletal muscle FA handling were determined by combining the

https://doi.org/10.1017/S0029665116003013 Published online by Cambridge University Press

forearm muscle balance technique with stable isotopes. [$^2H_2$]-palmitate was infused intravenously to label NEFA and VLDL–TAG in the circulation, whilst [U-^{13}C]-TAG was incorporated in a high-saturated FA-mixed-meal labelling chylomicron–TAG. Skeletal muscle biopsies were taken to assess intramuscular lipid content and the FSR. Systemic fasting and postprandial chylomicron and VLDL–TAG concentrations were comparable between the more pronounced IR *v.* the mild-IR subjects and in the IFG *v.* IGT subjects. Despite similar supply, muscle VLDL–TAG extraction was elevated in the high-IR group *v.* the mild-IR group and in the IGT *v.* IFG group, indicating that this increased muscle TAG extraction is a characteristic of the more pronounced insulin-resistant state in overweight and in pre-diabetic subjects. Another study comparing insulin-resistant and control subjects using the same dual-stable-isotope methodology could not confirm the findings of an increased muscle TAG extraction in IR, despite the fact that postprandial TAG concentrations were elevated[20]. The reason for these mixed results remains to be elucidated but it is important to emphasise that both studies confirm the apparent importance of TAG metabolism in IR.

The expression and activation of muscle lipoprotein lipase (LPL) plays a major role in muscle TAG extraction. Indeed, mice studies showed that deletion of LPL reduces lipid storage and increases insulin signalling in skeletal muscle[25]. Additionally, muscle-specific LPL overexpression induced muscle insulin resistance[26], while skeletal muscle LPL knockdown showed the reverse effect. The mechanism behind the earlier reported increased VLDL-extraction in IR remains to be elucidated, but may relate to a differential Apo composition. In a previous study, a higher ApoCII/apoCIII ratio of VLDL–TAG has been shown in diabetic patients as compared with a control group[27], which may lead to a higher affinity for lipolysis by skeletal muscle LPL. Nevertheless, the significance of a differential Apo composition in skeletal muscle lipid extraction remains to be determined. Noteworthy, it cannot be excluded that these findings are not only limited to VLDL–TAG extraction, but also extend to postprandial chylomicron–TAG extraction, but that our 4 h postprandial measurement period was too short to detect any significant effect. It has been shown that dietary FA appear in the VLDL–TAG from 2 to 3 h after meal ingestion, making it difficult to separate chylomicron- and VLDL–TAG in the late postprandial phase using the current stable isotope approach[8,28,29]. If so, other mechanisms like an impaired inhibitory action of insulin on LPL activity[30] may be an additional explanation. Indeed, in contrast to adipose tissue and liver, insulin infusion decreased skeletal muscle LPL activity in human subjects[31]. Furthermore, an increased FA uptake via membrane associated carrier proteins may be an explanation for an increased FA extraction. Interestingly, a 1·5-fold increase in CD36 protein content during insulin stimulation has been previously reported, which was positively associated with IR as measured during a hyperinsulinaemic–euglycaemic clamp[32]. Finally, other putative regulators of LPL activity such as ANGPTL4 may possibly contribute to the impairments in postprandial muscle FA handling. Indeed, muscle LPL activity is inhibited at posttranslational by ANGPTL4[33], but little is known on the physiological significance of this regulation *in vivo* in human subjects. A recent study showed that ANGPTL4 is secreted by human forearm muscle in postprandial conditions after a high-SFA meal, whilst plasma ANGPTL4 concentrations were not associated with *in vivo* skeletal muscle LPL activity after a high-SFA meal[34]. Nevertheless, although these findings do not suggest a major role of ANGPTL4 in the altered TAG extraction in, it remains to be determined to what extent circulating ANGPTL4 reflects ANGPTL4 activity at muscular level.

Skeletal muscle lipid turnover

When FA enter the myocyte they bind to cytoplasmic cystosolic FA binding protein for transport through the cell and they can be either directed towards the mitochondria for oxidation or towards storage in the muscle TAG stores in lipid droplets. Increased TAG synthesis, via up-regulation of lipogenic enzymes, has been related to protection against FA-induced IR in rodents and human subjects (as reviewed in[4]). Not much is known on muscle lipid turnover measured *in vivo* in human subjects in relation to IR. Bergman *et al.*[35] showed that the muscle TAG concentrations and its FSR were not altered in more insulin-resistant smokers as compared with non-smokers. Furthermore, the same group showed a reduced FSR of muscle TAG, higher TAG concentrations, a reduced oxidative capacity and an impaired peripheral insulin action in obese pre-diabetic subjects as compared with normal glucose tolerant controls[36]. Notably, these disturbances in muscle TAG metabolism were not found in women, indicating sex-related differences in muscle FA handling[37].

In the earlier indicated studies, where an increased VLDL–TAG extraction was reported in more pronounced IR in overweight and pre-diabetic subjects, also the content of skeletal muscle lipid metabolites, their FA composition and their FSR was assessed, using skeletal muscle NEFA as the precursor pool for lipid synthesis. Comparing the more pronounced overweight insulin-resistant subjects to the mild-IR subjects, showed an increased saturation of the skeletal muscle NEFA pool, possibly suggesting an increased retention and reduced metabolism of in particular SFA. In line, it was shown that the more pronounced insulin-resistant IGT subjects (either isolated or in combination with IFG) had a reduced saturation and fractional synthesis of the DAG and TAG pool, and a reduced expression of genes involved in oxidative metabolism as compared with the isolated IFG group, confirming that a reduced muscle lipid synthesis and turnover may be an important characteristic of the insulin-resistant muscle[11], (Fig. 1).

Dietary fat quality and skeletal muscle fatty acid handling

The previously and earlier reported disturbances in muscle FA handling and turnover in insulin-resistant conditions

https://doi.org/10.1017/S0029665116003013 Published online by Cambridge University Press

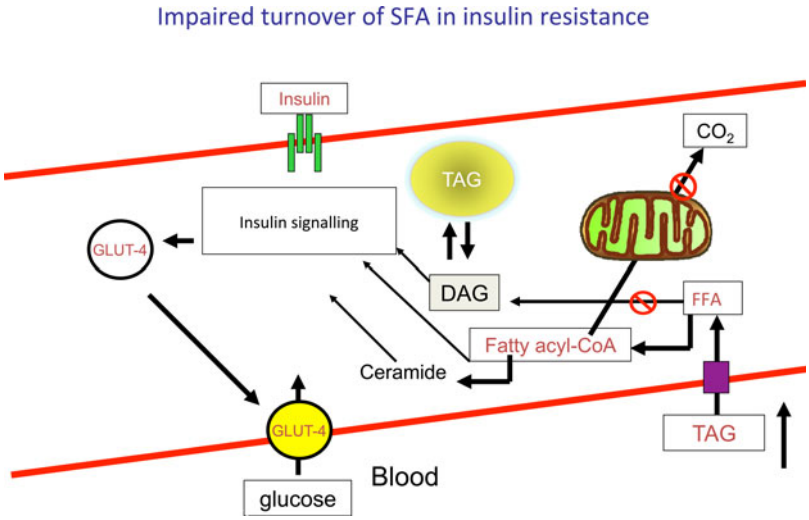

Fig. 1. (Colour online) Impaired muscle lipid turnover after a high fat, high saturated fat (SFA) meal in insulin-resistant subjects. The muscle of insulin-resistant subjects either in the overweight of prediabetic state is characterised by an increased postprandial (VLDL)–TAG extraction and a reduced fractional synthesis of muscle diacylglycerol (DAG) and TAG after a high fat, SFA meal and a reduced fasting transcriptional oxidative profile. We hypothesise that an increased proportion of the SFA is retained in the NEFA (FFA) pool, leading to a higher saturated fatty acyl-CoA content and an increased ceramide formation, which may in turn affect insulin sensitivity. Based on[11].

were shown after a high-SFA meal. There are indications that dietary fat quality may have an impact on pathways of FA handling and insulin sensitivity. Indeed, dietary intervention, including a reduction in SFA, has been shown to improve insulin sensitivity[38,39], possibly through effects on muscle lipid handling, although data are not consistent[40–43]. PUFA may reduce lipid overflow through inducing adipocyte differentiation thereby increasing lipid uptake as shown in a human preadipocyte cell line[44]. Additionally, it was shown in human muscle cell lines that SFA accumulate preferentially as DAG, whilst unsaturated FA are readily converted to TAG[45]. Also, a reduced fat oxidation was shown when diabetic myotubes were exposed to palmitic acid, whilst no differences were reported with oleic acid[46]. Based on the earlier study, it was hypothesised that a meal high in PUFA may acutely improve insulin sensitivity compared with a meal high in SFA in overweight insulin-resistant subjects. For this, ten obese insulin-resistant men consumed three high-fat mixed meals (61 E % fat), which were high in SFA (35·5 E %), MUFA (42·2 E %) or PUFA (34·8 E %), respectively. Fasting and postprandial forearm muscle FA processing were examined with the earlier indicated dual-stable-isotope approach in combination with the forearm muscle balance technique[12]. The high-PUFA meal significantly reduced TAG-derived skeletal muscle FA uptake, which was accompanied by higher postprandial insulin sensitivity, a more transcriptional oxidative phenotype, and an increased FSR of the DAG and TAG pool as compared with the high-SFA meal. These data indicate that the insulin-resistant muscle is characterised by both an increased muscle TAG extraction as well as a reduced muscle lipid turnover after in particular a high-SFA-mixed meal and not after a PUFA meal. Replacement of SFA by PUFA would therefore be protective against the development of IR.

Long-term dietary intervention manipulating diet composition

Lifestyle intervention, focused on both diet and physical activity is effective in the prevention of diabetes with a reduction in cumulative diabetes incidence of more than 50 % as shown in the European Diabetes Prevention Study[47] and the Diabetes Prevention Programme over 3–6 years[48]. In these studies, a low-fat, high-complex carbohydrate diet with a high-dietary fibre content was advised. Another dietary approach in the prevention of diabetes is the Mediterranean diet, rich in olive oil, which may provide cardiovascular benefits[49].

Manipulation of dietary fat quality by increasing the MUFA content or the *n*-3 or *n*-6 long-chain PUFA content of the diet in intervention studies has not shown consistent results on insulin sensitivity[40–43]. Based on the earlier findings on FA handling and insulin sensitivity in different prediabetic states, it can be speculated that effectiveness of dietary fat manipulation may depend on initial metabolic phenotype.

In line, in the CORDOPREV-DIAB study, the low-fat and Mediterranean dietary patterns were compared with respect to tissue-specific IR and β-cell function in cardiovascular patients not treated for diabetes (*n* 642, analysis at baseline and at 2 years follow-up)[50]. Although both diets improved insulin sensitivity, there were distinct differences based on the IR phenotype. More specifically, the

https://doi.org/10.1017/S0029665116003013 Published online by Cambridge University Press

low-fat diet resulted in a higher increase in disposition index (an estimation of β-cell function: insulin secretion adjusted for peripheral insulin sensitivity) in patients with liver IR, whilst the Mediterranean diet resulted in a higher increase in disposition index and insulinogenic index (an estimation of insulin secretion) in patients with skeletal muscle IR. In addition, a recent *post hoc* analysis in the European project LIPGENE, focused on the study of dietary fat quantity and quality in the metabolic syndrome, showed that insulin-resistant individuals were more susceptible to a health effect from the substitution of a high-saturated fat diet by either high MUFA and high (complex) carbohydrate (with added *n*-3 PUFA) diets. In addition, metabolic syndrome individuals without IR were more sensitive to the detrimental effects of high-saturated fat intake[51]. These data suggest that dietary prevention or treatment may require a more personalised or sub-group-based approach to become most effective. Nevertheless, this remains to be confirmed in prospective dietary intervention studies specifically designed to address baseline phenotype in relation to intervention success. Interestingly, a recent study by Zeevi *et al.*[52] showed that despite high interpersonal variability in post-meal glucose, personalised diets created with help of an algorithm, including dietary habits, physical activity and gut microbial composition, is successful in lowering blood glucose concentrations. This indicates that advances in detailed phenotyping including -omics methodologies, advances in 'quantify self' methods for dietary intake, blood glucose patterns and other physiological parameters in daily life may yield new opportunities for more personalised and sub-group-based approaches. At first, more prospective evidence has to be derived for the plausibility and urgency of this approach in daily life.

Financial Support

None.

Conflicts of Interest

None.

Authorship

The author had sole responsibility for all aspects of preparation of this paper.

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https://doi.org/10.1017/S0029665116003013 Published online by Cambridge University Press