The global epidemic of type 2 diabetes (T2D) and its co-morbidities threatens to overwhelm public health services and urgent patient intervention is necessary. A review of mainly randomised controlled trials investigating the reduction of biochemical T2D risk markers through fasting or caloric restriction (CR) found that in T2D or where baseline fasting glucose or HbA1c were elevated, there were significant improvements in fasting glucose and HbA1c, while fasting insulin and insulin resistance may show improvement regardless of condition or baseline levels. There may, however, be ethnic differences, with a clear positive correlation found only in Caucasians. Intermittent CR (i.e. non-continuous periods of fasting) is at least as effective as isocaloric continuous CR, while CR of 400-800 kcal/day is possibly more effective than higher levels for reducing fasting glucose and HbA1c. Time restricted feeding also shows promise but there are few human studies. The findings suggest that the optimum regimen to reduce biochemical risk markers for T2D is an intermittent fasting programme employing a very low-calorie diet with the longest possible number of consecutive days of fasting. The addition of liquid meal replacements, low carbohydrate CR and supplementation of vitamin D, ω-3 PUFAs and L-carnitine may also be of benefit.

Keywords: Type 2 diabetes, metabolic syndrome, glucose, insulin, insulin resistance, caloric restriction, fasting

INTRODUCTION

There is now a global epidemic of type 2 diabetes (T2D), metabolic syndrome and obesity, which threatens to overwhelm public health services, not just in terms of the conditions themselves but also because of their co-morbidities, such as cardiovascular disease (Sookian and Pirola, 2011; Harris, 2013), dementia, breast cancer (Loveman et al, 2011) and increased mortality (Deedwania and Gupta, 2006). The International Diabetes Federation estimated in 2013 that the global prevalence of T2D in adults aged 20-79 was 8.3% (382 million people); the number is expected to more than double by 2020 and rise beyond 592 million by 2035 with a 10.1% global prevalence (International Diabetes Federation, 2013; Lin and Sun, 2010). The Middle East and North Africa have the highest prevalence of diabetes (10.9%), although the Western Pacific region is a close contender (International Diabetes Federation, 2013; Dabelea et al, 2014). Similarly, it has been estimated that 20% of adults in the Western world have metabolic syndrome (Kaur, 2014), while 30-40% of the population are reported to be insulin resistant (Bonora, 2005). T2D incidence is occurring at considerably younger ages, with a 30.3% overall increase in T2D in children and adolescents between 2001 and 2009 (Dabelea et al, 2014; Ervin, 2009); the American Diabetes Association now recommends screening of overweight children and adolescents (American Diabetes Association, 2000).

Diabetes mellitus is a metabolic disease in which chronic hyperglycaemia results from defects in insulin secretion and/or insulin action, which can manifest in most tissues but mainly in skeletal and cardiac muscle, adipose tissue and the liver. It is diagnosed using either a measure of plasma glucose or HbA1c, together with symptoms of hyperglycaemia, although it has been observed that ethnicity may affect the threshold values for diagnosis, particularly in the Chinese, Japanese, Egyptians and Pima Indians. In healthy individuals without insulin resistance, the pancreatic β-cells secrete insulin in response to elevated glucose concentrations in the circulation following...
digestion. Insulin binds to its receptors, found on most cells, triggering the translocation of the insulin-regulated glucose transporter GLUT-4 to the surface of the cell membrane to facilitate the transport of glucose into the cell for energy, thereby triggering a reduction in circulating glucose concentrations; skeletal muscle is responsible for most of the insulin-mediated glucose uptake in non-obese subjects. When the glucose has entered the cells and the body detects that blood glucose levels have returned to a safe range, insulin production is inhibited in a healthy negative feedback loop. The balance between glucose storage and mobilisation is maintained by the opposition of the two regulatory hormones insulin and glucagon, secreted by the β- and α-cells of the pancreas, respectively. (Chiu et al., 2007; Wang and Guanyu, 2014; Schinner et al., 2005; Kharroubi and Darwish, 2015)

However, where blood glucose remains too high (hyperglycaemia), insulin will be continually secreted to the point where the insulin receptors become overwhelmed or blocked and fail to respond normally, and plasma insulin levels will rise (hyperinsulinaemia). The receptors will lose sensitivity, a condition known as insulin resistance, which represents a positive feedback loop since there is no intervention from the body which can break the cycle. Insulin resistance increases the demand for insulin in target tissues but the pancreatic β-cells are unable to secret sufficient insulin to meet this demand and their gradual destruction ensues. There is also some suggestion that with the development of T2D, the pancreatic α-cells may also become dysregulated, resulting in inappropriately high glucagon secretion, which may contribute to the hyperglycaemia (O’Neill and O’Driscoll, 2015). In healthy individuals, the liver would detect the hyperglycaemia and reduce its glucose production but this may not occur in insulin resistant individuals, who will ultimately develop such severe hyperglycaemia that type 2 diabetes (T2D) is diagnosed and anti-diabetic medications are required. (Chiu et al., 2007; Wang and Guanyu, 2014; Schinner et al., 2005; Kharroubi and Darwish, 2015)

The development of T2D is multifactorial, with contributions from genetics, diet, lifestyle, environmental toxins and many others. Although genome-wide studies have shown that single nucleotide polymorphisms (SNPs) can explain only 20% of the heritability of T2D, several other related genetic candidates have appeared, such as SNPs in genes regulating DNA methylation, immune function, inflammation, cellular stress and defects in fatty acid and amino acid metabolism, which all appear to have a bearing on T2D development (Nicoll, 2017; Soeters et al., 2009). Furthermore, recent genetic studies have demonstrated that severe insulin resistance syndromes (primary insulin signalling defects, lipodystrophies and other syndromes) can predispose to the development of T2D (Das et al., 2007). Additional factors include endothelial dysfunction and lipotoxicity; although more commonly associated with cardiovascular disease, endothelial dysfunction may play a role in the pathogenesis of T2D through hyperglycaemia damaging the endothelium, combined with impaired insulin signalling in endothelial cells and the secretion from adipose tissue of pro-inflammatory adipokines and large quantities of free fatty acids, which induce lipotoxicity in a number of tissues (Trepanowski et al., 2017; Razny et al., 2015).

Insulin resistance is a key risk factor for obesity, metabolic syndrome, T2D, non-alcoholic fatty liver disease (NAFLD) and cardiovascular disease (CVD). Although largely without symptoms, it is nevertheless a complex condition affecting all tissues which take up glucose for energy (mainly the muscles and liver) but also impacts the liver’s ability to produce and transport glucose and to store excess glucose as glycogen, resulting in the conversion of the excess to triglycerides (de novo lipogenesis). (Kharroubi and Darwish, 2015; Nicoll, 2017) Despite insulin resistance being an important risk factor for T2D, treatment for T2D focuses mainly on glycaemic control with diet and lifestyle recommendations. This may be because reversing insulin resistance, once well-established, is a major problem for physicians and patients, since there are no pharmaceutical drugs which can help.

Instead, fasting, caloric restriction (CR) and time restricted feeding (TRF) have shown promise as effective interventions for weight loss (Georg Jensen et al., 2012) and may also be effective in lowering the biochemical risk markers for T2D: blood glucose, HbA1c, insulin and insulin resistance. Caloric restriction may be either continuous (CCR) or intermittent (ICR), with alternate day fasting (ADF) being a common variant of ICR, comprising zero calories or very few calories. This review investigates those studies which assess the impact of fasting, CR and TRF on biomarkers for T2D and attempts to find the optimum regimen for successfully lowering T2D risk markers. The papers discussed will almost exclusively comprise findings from randomised controlled trials (RCTs) since these are likely to be of higher quality, but other studies are mentioned as necessary or to add clarification. The many studies of exercise alone as a means of improving biomarkers are too numerous to discuss here but where exercise is an arm of a fasting or CR trial, then its effect is described. The studies are grouped by effect firstly on blood glucose, then HbA1c, insulin and finally insulin resistance, and are divided according to whether the subjects have T2D or not.

Impact of CR on fasting blood glucose and glucose tolerance

Subjects without metabolic syndrome or T2D

In normal weight or overweight subjects with fasting plasma glucose well within the reference range, fasting or CR usually has no effect on fasting glucose (Soeters et al., 2009; Das et al., 2007; Trepanowski et al., 2017). Even in
younger obese subjects, provided fasting blood glucose is not towards the upper limit of the reference range, CR often has no effect on fasting plasma or serum glucose (Razny et al, 2015; Georg Jensen et al, 2012, Harvie et al, 2011; Metzner et al, 2011; Harvie et al, 2013), although zero calorie ADF for eight weeks was able to reduce fasting glucose from baseline, whereas CCR could not; nevertheless, after 32 unsupervised weeks the difference from baseline was no longer significant (Catenacci et al, 2016). In obese postmenopausal females, however, ICR was again successful in lowering fasting plasma glucose relative to CCR over 20 weeks, but after one year both groups showed a significant reduction (Arguin et al, 2012).

Where baseline mean fasting glucose is on the upper threshold of the reference range and some subjects have T2D or pre-diabetes, some trials may show a reduction in fasting glucose. Three RCTs by Weiss et al investigated CR with or without exercise and found that in overweight or obese subjects with borderline elevated glucose who were randomised to a 20% energy deficit with or without exercise or exercise alone, there was a significant decrease in fasting plasma glucose in the two CR groups only but no difference between groups (Weiss et al, 2016). An almost identical earlier RCT by the same team had shown that CR plus exercise was more effective at improving fasting glucose than either intervention alone, although glucose tolerance improved significantly in all intervention groups with no difference between groups, and among pre-diabetics a similar percentage from each intervention group became normo-glycaemic (Weiss et al, 2015). These results echo Weiss et al's 2006 study, which showed that in non-obese subjects the glucose tolerance improvement with CR or exercise after 12 months was significant but was similar in the two intervention groups, while fasting glucose reduced significantly in the CR but not the exercise or control groups, although this reduction was not statistically significant after adjusting for baseline values (Weiss et al, 2006). Because not all studies adjust for baseline values, comparison of results can be problematic. Additionally, Nicklas et al showed that in overweight or obese postmenopausal females randomised to CR (400 kcal/day energy deficit), CR + moderate intensity aerobic exercise or CR + vigorous intensity exercise for 20 weeks, fasting and 2-hour glucose was significantly reduced but there was no advantage to aerobic exercise of any intensity over CR (Nicklas et al, 2006).

Davoodi et al randomised overweight or obese subjects with borderline elevated blood glucose to four weeks CR (55% intake restriction) or a calorie sparing diet (CSD) with 45% intake restriction, for six weeks with a four week follow-up (Davoodi et al, 2014). Plasma glucose decreased to a significant extent in both groups and remained significantly decreased from baseline after four weeks follow-up but was significantly lower in the CSD group vs the CR group; mean plasma glucose in both groups was no longer elevated after the intervention phase or at follow-up. Likewise, obese insulin-resistant subjects with borderline elevated mean fasting plasma glucose underwent CCR (1000 kcal/day energy deficit) until they lost 7% of body weight; after 48 hours and 11 weeks both groups had significantly reduced plasma glucose. (Kirk et al, 2009)

Subjects with metabolic syndrome or T2D

Generally, overweight or obese subjects with metabolic syndrome or T2D and elevated fasting blood glucose who undergo CR show a significant decrease in both weight and blood glucose concentrations in trials lasting 12 weeks, 16 weeks and six months (Ruggenenti et al, 2016; Choi et al, 2013; Pascale et al, 1995; Wycherley et al, 2008), although after one year's unsupervised follow-up, blood glucose and weight had returned to baseline levels (Pascale et al, 1995). Surprisingly, even overweight or obese subjects with metabolic syndrome but fasting blood glucose within the reference range, who underwent two days of 700-800 kcal/day CR followed by five days of 200 kcal/day CR also experienced a significant reduction in fasting blood glucose (Zhang et al, 2014). Blood glucose was similarly reduced where subjects were randomised to moderate high protein CR with or without aerobic exercise for 12 weeks, with no difference between groups (Wycherley et al, 2008). One study, however, showed no effect of 1200-1400 kcal/day CR for 10 days on fasting plasma glucose, possibly because the study duration was too short, although glucose tolerance improved significantly (Molfino et al, 2010).

The results of comparing moderate CR with a very low calorie diet (VLCD) are mixed. Williams et al showed that in obese type 2 diabetics, the greatest extent of CR (400-800 kcal/day vs 1500-1800 kcal/day) produced the greatest reduction in fasting plasma glucose after three weeks but there was no difference at the end of 20 weeks, although changes in fasting plasma glucose at the end of the third week predicted the 20 week improvement (Williams et al, 1998). Similar results were seen when obese subjects with T2D were randomised to moderate CR or very low calorie diet (VLCD) for eight weeks followed by moderate CR for eight weeks in both groups, followed up after one year. The VLCD produced a significantly greater decrease in fasting glucose after 16 weeks but in this RCT the improvement was maintained at one year (Wing et al, 1991).

Because CR inevitably leads to weight loss, Wing et al point out that it is difficult to design studies to separate out the effects of caloric restriction and weight loss. For this reason, their study equalised the degree of weight loss and then equalised the caloric restriction by randomising obese subjects to 400 or 1000 kcal/day with the goal of attaining 11% reduction in baseline body weight, when all groups then consumed 1000 kcal/day for 15 weeks. Once subjects had lost 11% of body weight, those in the 400 kcal/day group had significantly lower fasting glucose, and
their improved glycaemic control occurred after just three days. When caloric intake was equalised, weight loss was also similar between groups, although glycaemic control worsened in those originally in the 400 kcal/day group and after 15 weeks fasting glucose was significantly higher than in the group which had consumed 1000 kcal/day throughout. The authors concluded from this that both the degree of CR and the magnitude of weight loss have independent effects on glycaemic control. Although this RCT certainly demonstrated that CR independently affects fasting blood glucose, the fact that weight loss was comparable all the time that CR was comparable does not in fact show that weight loss independently influences fasting blood glucose. (Wing et al, 1994)

**Type of diet**

The type of diet generally has no effect on fasting blood glucose where it is not elevated at baseline, as was seen in RCTs which found no difference between a high or low glycaemic load in younger overweight subjects with fasting plasma glucose within normal range (Das et al, 2007), between overweight or obese females randomised to CCR with or without meal replacement at two meals per day (Metzner et al, 2011) or ICR with low carbohydrate compared with ICR with unlimited protein and fat or Mediterranean-type (relatively high protein and fat, low glycaemic index carbohydrate) CCR (Harvie et al, 2013). Nevertheless, a meal replacement study in obese females with elevated fasting glucose, who were randomised to fasting for one day per week and mild (25% energy deficit) caloric restriction for the remaining six days, with or without a liquid diet, for eight weeks showed that fasting glucose was significantly reduced only in the liquid group (Klempel et al, 2012).

Likewise, in obese insulin-resistant subjects with borderline mean fasting plasma glucose who were randomised to CCR (1000 kcal/day energy deficit) with a low fat, high carbohydrate diet or a high fat, low carbohydrate diet, after 48 hours the decrease in plasma glucose was significantly greater in the low carbohydrate diet group but after 11 weeks there was no difference between groups, although hepatic glucose production rate showed a significantly greater improvement in the low carbohydrate group after both 48 hours and 11 weeks (Kirk et al, 2009). An RCT of CR, with or without additional fat restriction for 16 weeks showed that in obese females with T2D and severely elevated blood glucose compared to females with a family history of T2D and mildly elevated blood glucose, there was a significant reduction in blood glucose in all groups, with no difference between them, although after one year blood glucose had returned to baseline levels. (Pascale et al, 1995)

**Impact of CR on HbA1c**

**Subjects without metabolic syndrome or T2D**

Overweight or obese females with serum HbA1c within the reference range were randomised to 25% CCR or ICR for three months followed by one month of weight loss maintenance. After three months and an additional unsupervised month, there was no difference in HbA1c from baseline or between groups. (Harvie et al, 2013) Similarly, overweight or obese females with serum HbA1c within normal range were randomised to CCR (1200 kcal/day) but after 12 weeks, there was no difference in serum HbA1c from baseline (Metzner et al, 2011).

**Subjects with T2D**

Generally, when subjects with T2D and elevated mean HbA1c undergo some form of CR, HbA1c is significantly improved after 12 weeks, 16 weeks or six months (Ruggenenti et al, 2016; Choi et al, 2013; Pascale et al, 1995; Wycherley et al, 2008; Ash et al, 2003); the addition of exercise to the CR had no additional effect (Wycherley et al, 2008). After one year unsupervised, HbA1c returned to baseline (Pascale et al, 1995). Ash et al calculated that a 1% reduction in HbA1c was associated with a 6.5% weight reduction, and noted that subjects who attained normal glycaemia had significantly greater weight loss than subjects whose HbA1c level remained >6%, although at 18 months follow-up, HbA1c was similar to baseline levels in the subject group as a whole (Ash et al, 2003). Furthermore, it appears that a VLCD is associated with a significantly greater reduction in HbA1c than moderate CR after one year, but not after 20 weeks (Wing et al, 1991). However, where hyperglycaemia is well controlled by medication, seven day Buchinger fasting (300kcal/day in liquid form) may produce no significant change in HbA1c (Li et al, 2017).

A study by Williams et al randomised obese diabetics to 1500-1800 kcal/day for 20 weeks (Group 1), a very low calorie diet (400-800kcal/day) for five consecutive days in four specific weeks and the 1500-1800 kcal/day diet for the remainder of the time (Group 2) or the very low calorie diet for five consecutive days in week 2 and then for one day per week for 15 weeks and the 1500-1800 kcal/day diet for the remainder of the time (Group 3). There was no difference in HbA1c between groups at 10 or 20 weeks but a significantly greater number of subjects had normalised HbA1c at 20 weeks in group 2 compared to group 1. Using logistic regression, there was no significant effect of weight loss on normalisation of HbA1c, but changes in fasting plasma glucose at the end of the third week predicted the 20 week improvement in HbA1c. (Williams et al, 1998) In this study, it seems that it is not the extent of CR alone or the total number of days of CR that made the difference but possibly the fact that the greatest number of subjects normalised HbA1c in the group with the greatest number of consecutive fasting days. Since glycaemic control is the primary goal of therapy in T2D, this may provide useful guidance for future trials.

**Type of diet**

Where HbA1c is within the reference range, 25% CR comprising intermittent restriction with very low
carbohydrate for two days per week, the same conditions with unlimited protein and fat or Mediterranean-type CCR for three months was not associated with reduction of HbA1c from baseline and there was no difference between groups (Harvie et al., 2013). Similarly, a trial of high carbohydrate, low fat CCR (1200 kcal/day) for three meals per day with or without meal replacement at two meals, in the form of shake, soup or bar, for 12 weeks showed no difference in serum HbA1c from baseline in either group or between groups (Metzner et al., 2011). However, if HbA1c is elevated at baseline in overweight or obese females with T2D, high carbohydrate CR for 12 weeks was effective in reducing fasting HbA1c compared to no CR (Choi et al., 2013), whereas CR vs CR with additional fat restriction for 16 weeks was associated with a significant reduction in HbA1c from baseline but with no difference between groups (Pascale et al., 1995)

Impact of CR on fasting blood insulin and insulin secretion

Subjects without T2D

Most studies show that CR or fasting in overweight or obese subjects is associated with significantly reduced fasting insulin (Trepanowski et al., 2017; Weiss et al., 2016; Heilbronn et al., 2006). However, at two, six and 12 months, there was no difference between ADF and CCR (Trepanowski et al., 2017; Catenacci et al., 2016) although one study showed that ICR was more effective at lowering serum insulin than the isocaloric Mediterranean-type CCR after three months, indicating that it is not caloric restriction per se which makes a successful insulin-lowering diet (Harvie et al., 2013). After an unsupervised period, fasting blood insulin normally returned to baseline values (Catenacci et al., 2016). Where some of the subjects have metabolic syndrome or pre-diabetes, even though fasting insulin is within normal range, both ICR and CCR were shown to induce significant reductions in mean serum fasting insulin after six months but the reduction was significantly higher in the ICR group (Harvie et al., 2011). Where CR is employed, with or without exercise, it is generally found that exercise adds nothing to the effect of CR, even high intensity exercise (Weiss et al., 2016; Nicklas et al., 2009).

One study showed that baseline insulin was only significantly reduced when the calories were provided in liquid form, although the authors consider that this may have been due to a greater degree of adherence in the liquid diet group, who received portion-controlled liquid breakfasts and lunches rather than estimation of solid food, which is known to be faulty in obese subjects (Klempel et al., 2012). In obese insulin resistant subjects, randomised to CCR with a low fat, high carbohydrate diet or a high fat, low carbohydrate diet, after 11 weeks there was a significant decrease in fasting insulin only in the low carbohydrate diet (Kirk et al., 2009). However, where CR is employed with a low or high glycaemic load, there was a significant reduction in fasting insulin from baseline at six and 12 months, but the difference between groups was not significant (Das et al., 2007).

Subjects with T2D or metabolic syndrome

In overweight or obese Taiwanese and Chinese subjects with metabolic syndrome, 1500 kcal/day CR for 12 weeks or increased restriction produced a significant reduction in fasting insulin, with no difference between groups (Zhang et al., 2014; Su et al., 2015). In subjects with non-Chinese origin, there was similarly no difference in the significant reduction in fasting plasma insulin between 1500-1800 kcal/day or 400-800 kcal/day alternating with the higher calorie diet for 20 weeks (Williams et al., 1998). In the Wing et al. study, in which baseline fasting insulin was elevated or borderline elevated, both groups significantly reduced fasting insulin but there was no difference between subjects on 1,000 or 400 kcal/day after 11% of baseline body weight had been lost, suggesting that reduction in fasting insulin is unaffected by extent of CR (Wing et al., 1994). This result is echoed in RCTs of obese subjects with T2D randomised to 25% CR or no CR for six months (Ruggenenti et al., 2016) or seven day Buchinger fasting (Li et al., 2017). Where obese subjects with T2D were randomised to CR with or without aerobic exercise for 12 weeks, fasting insulin was significantly reduced in both groups, with exercise adding no benefit to the CR (Wycherley et al., 2008).

Impact of CR on insulin sensitivity or resistance

Subjects without T2D or metabolic syndrome

Even where subjects are not insulin resistant, fasting or CR can have a significant effect on improving insulin sensitivity during the weight loss programme. When obese adults were randomised to either zero-calorie ADF or CCR for eight weeks, followed by a further 24 week unsupervised period, after both eight and 24 weeks there was no difference between the groups in improvement in insulin sensitivity from baseline (Catenacci et al., 2016). Nevertheless, in overweight females randomised to 25% CCR or ICR, insulin resistance reduced only in the ICR group during the weight loss programme, echoing the reduction in body fat, but after one month of weight maintenance, there was no difference between groups (Harvie et al., 2013). Similarly, in insulin resistant subjects, it appears that ICR or ADF may be as effective as CCR for lowering HOMA-IR after six months’ intervention and a further six months unsupervised (Trepanowski et al., 2017).

The type of diet during CR also has little bearing on results after the weight maintenance programme, although a low carbohydrate diet is more effective during the weight loss phase. Overweight females were randomised to 25% CCR, ICR with carbohydrate restriction or ICR plus unlimited protein and fat but an effective energy restriction of 15%, for three months plus one month weight maintenance. At three months, insulin resistance reduced
with both ICR diets but not with CCR and the reduction was significantly greater with the low carbohydrate ICR relative to CCR alone; at four months, there was no difference between the groups. This indicates that it is not energy reduction per se which makes a successful insulin-lowering diet. (Harvie et al, 2013) When overweight or obese premenopausal females, some with metabolic syndrome, were randomised to Mediterranean-type 25% CCR or ICR (75% energy deficit), with very low carbohydrate, for two days per week followed by the Mediterranean-type diet for five days per week, HOMA-IR was reduced to a significantly greater extent in the ICR group compared to the CCR group after six months (Harvie et al, 2011). Another study found that in subjects randomised to CCR (1000 kcal/day energy deficit) with a low fat, high carbohydrate diet or a high fat, low carbohydrate diet until they lost 7% of body weight, HOMA-IR improved after 48 hours in both groups and did not change significantly thereafter up to 11 weeks but the reduction was significantly greater in the low carbohydrate group; hepatic and skeletal muscle insulin sensitivity also showed a significantly greater improvement in the low carbohydrate group (Kirk et al, 2009).

As in previous studies reviewed, the addition of exercise does not improve any reduction in insulin resistance through CR alone. Overweight adults were randomised to control, 25% CR, 12.5% CR + exercise or 15% weight loss by low calorie diet. At baseline, fat cell size was the strongest determinant of insulin sensitivity, which was significantly increased in the CR + exercise and low calorie diet groups but was not significantly different between groups. (Larson-Meyer et al, 2006) Similarly, in non-obese subjects randomised to exercise, CR or control for 12 months, the insulin sensitivity index increases were similar in the two intervention groups (Weiss et al, 2006), while randomisation of overweight subjects to 20% CR, exercise or healthy lifestyle guidelines for a year, resulted in a significant reduction in HOMA-IR in the CR and exercise groups only (Fontana et al, 2007). Similarly, in overweight subjects randomised to 6-8% weight loss by using 20% energy deficit CR, exercise or the combination, there was a 70% reduction in log HOMA-IR but reductions were similar in all groups (Weiss et al, 2016).

**Subjects with T2D or metabolic syndrome**

As might be expected, fasting and CR was also effective in lowering insulin resistance in subjects with T2D or metabolic syndrome. Overweight South Korean females were randomised to high carbohydrate CR or no intervention for 12 weeks; HOMA-IR was significantly decreased in the CR group (Choi et al, 2013). The study by Wing et al, in which obese subjects were randomised to 400 or 1000 kcal/day until they lost 11% of body weight, showed that insulin sensitivity was improved in both groups but those on the 400 kcal/day diet had significantly improved insulin sensitivity compared to those on the higher calorie diet. Once both groups consumed the same degree of CR for a further 15 weeks they lost a similar amount of weight but the improvement in insulin sensitivity was not statistically significant (Wing et al, 1994). Exercise again has no impact over and above CR, as seen in when subjects were randomised to moderate CR with or without aerobic exercise for 12 weeks; insulin resistance was significantly reduced in both groups with no difference between groups (Wycherley et al, 2008).

**Impact of CR with or without additional supplementation on risk markers for T2D**

The most commonly tested supplement is ω-3 polyunsaturated fatty acids (PUFAs), which have been shown to aid weight loss (Nicoll, 2017). Obese subjects with insulin resistance but fasting plasma glucose within the reference range, were randomised to a 1200-1500 kcal/day high carbohydrate diet with or without 1.8 g/day omega-3 PUFAs (DHA:EPA 5:1) for three months. Neither group showed a significant change from baseline or between groups for fasting plasma glucose but fasting plasma insulin and insulin resistance decreased significantly in the omega-3 PUFA group only. (Razny et al, 2015) However, a Taiwanese study randomised overweight or obese insulin resistant females with metabolic syndrome and clearly elevated blood glucose to 1500 kcal/day CR, CR with meal replacement, CR with 2130mg/dy ω-3 fish oil (1280 mg of EPA and 850 mg of DHA) or CR with meal replacement and fish oil. Fasting insulin concentrations and insulin resistance were significantly improved in all groups but fasting and postprandial glucose concentrations were significantly reduced and attained normal range only in subjects in the two groups containing fish oil. The metabolic syndrome Z-score was significantly improved in all groups but showed a 1.5-fold greater improvement in the supplementation groups relative to CR alone and metabolic syndrome recovery rate exceeded 60% in all groups except CR alone. Because the Z-scores were positively correlated with inflammatory markers, the authors suggested that ω-3 fish oils were beneficial due to their anti-inflammatory effect. (Su et al, 2015)

L-carnitine is also commonly recommended with fasting or CR to prevent loss of lean mass and it can also enhance glucose utilisation. Adults with T2D or metabolic syndrome were randomised to CR of 1200-1400 kcal/day with high carbohydrates, with or without 4g/day oral L-carnitine for 10 days; oral glucose tolerance tests improved in both groups with no significant difference between groups. Fasting plasma glucose did not reduce significantly and was not different between groups but fasting plasma insulin and insulin resistance significantly decreased from baseline with L-carnitine only. (Molfino et al, 2010) Similar results were seen with overweight or obese Chinese subjects with metabolic syndrome, but mean baseline blood glucose and insulin within normal range, who were randomised to fasting with or without L-carnitine infusion for one week. Fasting blood glucose, insulin and insulin...
resistance decreased significantly in both groups, with no difference between groups for change in glucose or insulin resistance but the L-carnitine group had significantly greater insulin reduction; possibly insulin resistance would also have reduced significantly if the trial had continued for longer than one week. (Zhang et al, 2014) These two studies suggest that L-carnitine does not aid fasting in the lowering of blood glucose but has a significant effect on lowering fasting blood insulin.

One study investigated CR with or without 2,500IU/d vitamin D3 for 6 weeks in overweight or obese postmenopausal females with mean baseline 25(OH)D of 62 nmol/l (24.8ng/ml). Both weight loss and vitamin D were associated with improved serum glucose, insulin and insulin resistance. (Sukumar et al, 2015)

Impact of CR or bariatric surgery on T2D and its risk markers

In a number of comparison studies, very low calorie diets (500 kcal/day) generally appear to be equally as effective as bariatric surgery, normally Roux-en-Y gastric bypass (RYGB), in lowering weight, fasting glucose and insulin and improving glycaemic control, insulin resistance and β-cell function in patients with T2D and/or morbid obesity during the two or three weeks following surgery and after several months unsupervised (Jackness et al, 2013; Campos et al, 2010; Lips et al, 2014; Lingvay et al, 2013; Mitterberger et al, 2010; Isbell et al, 2010). A few studies even showed that CR was associated with a greater decrease in fasting insulin that RYGB (Lips et al, 2014). Following surgery, the improvement in glycaemic control appears to occur prior to major weight loss, suggesting that it is not due to weight loss, although improvement in peripheral glucose uptake is observed only after substantial weight loss had occurred (Campos et al, 2010). Nevertheless, one study found that two weeks after the intervention, insulin and glucose exhibited a more rapid decrease in fasting insulin that RYGB even showed a measured outcome, insulin and glucose also have reduced significantly if the trial had continued for longer than one week.

This review found that there may be an association of T2D biomarkers and weight. Since it is clear that fasting and CR can significantly lower weight (Nicoll et al, 2017), and have in fact effectively lowered weight in all the RCTs in this review where it was a measured outcome, it may be instructive to look at any relationship between these T2D biomarkers and weight. This review found that there may be an association of HbA1c with weight loss, with one study finding that a 1% reduction in HbA1c was associated with a 6.5% weight reduction. Subjects who attained normal glycaemia had significantly greater weight loss than those whose HbA1c remained >6%. Fasting insulin also appears to be associated with weight loss, with those achieving the greatest weight loss also achieving the greatest reduction in fasting insulin, suggesting that it is independent of the extent of CR. Insulin resistance and fasting plasma glucose, however can be affected by both weight loss and extent of CR independently.

These findings contrast with a number of studies, mainly dating from the 1990s, which have suggested that insulin resistance, far from being associated with weight gain, may in fact be associated with resistance to weight gain by increasing central nervous system insulin signalling which suppresses food intake. Several of these studies were carried out on Pima Indians (Swinburn et al, 1991; Valdez et al, 1994), although some used other ethnicities (Sigal et al, 1997; Hoag et al, 1995). However, Sigal et al pointed out that the Pima Indians tended to be more obese and more insulin resistant at baseline and there were a number of differences in methodology between these and other studies (Sigal et al, 1997). Weyer et al note that Pima Indians are known to have an increased pancreatic parasympathetic drive, as measured by pancreatic polypeptide as a surrogate marker for vagal tone, which could result in hypersecretion of insulin and contribute to their increased tendency to develop obesity and T2D (Weyer et al, 2001). Nevertheless a 2004 study of over 3,300 postmenopausal females, of various ethnicities, showed that in multivariate analysis, baseline insulin resistance and insulin concentrations were independent predictors of increases in weight in the sample as a whole, but when stratified by ethnicity, the relationship held in Caucasians but was not statistically significant in Hispanics or Asian/Pacific Islanders, whereas there was no predictive ability of baseline insulin resistance in African Americans. Furthermore, there was a significant interaction of insulin resistance with body mass index (BMI), making the effect of insulin resistance on weight gain more pronounced in leaner women. (Howard et al, 2004) Possibly this study holds the key to the earlier results, since African Americans are known to be more at risk for T2D and CVD, even though many of the key risk markers are not elevated.

The series of studies by McLaughlin et al, who investigated CR for weight loss in overweight or obese female non-diabetics, also produced some interesting results. In their 1999 study, the authors stratified their subjects by ability to lose 1% of ideal body weight per week but found no correlation between weight loss ability and baseline insulin resistance over the short term (McLaughlin et al, 1999). In a later study, they stratified their subjects by baseline insulin resistance and again, after four months’ CR, there was no difference in weight loss between groups, although among the insulin-resistant, weight loss was associated with significantly decreased fasting glucose and insulin, as well as insulin resistance, while among the insulin-sensitive, fasting glucose, insulin and insulin sensitivity were unchanged. The authors point out that their ability to recruit insulin-sensitive obese subjects to the study calls into question the prevailing view that all obese individuals are insulin-resistant. (McLaughlin et al, 2001)
However, since insulin resistant subjects in this study had significantly higher baseline fasting glucose and insulin than insulin sensitive subjects, in whom they were normal, this result is not remarkable and merely suggests that CR can bring about improvement in metabolic risk factors, including weight, where it would be of metabolic benefit, but has no effect where improvement is not required i.e. where risk factors are already in normal range. This in fact concurs with the findings of this review, namely that where fasting blood glucose and HbA1c are not elevated, CR has no significant effect. The McLaughlin et al study duration was relatively short but it is reasonable to suppose that had CR been continued among the insulin resistant subjects, fasting glucose and insulin, as well as insulin sensitivity, would eventually have normalised, after which there would presumably have been no further decrease, since none was necessary (McLaughlin et al, 2001).

McLaughlin et al's 2004 study found a significant positive correlation between insulin resistance and BMI, although it was also the case that 16% of subjects in the most insulin resistant tertile were of normal BMI (McLaughlin et al, 2004). There was also a correlation between insulin resistance and fasting plasma glucose and insulin and the three hour glucose and insulin response, as well as a significant association between tertiles of BMI and fasting plasma glucose and insulin. Similarly, in their 2008 study, insulin-resistant obese subjects underwent CR for 16 weeks and were followed up after 29 unsupervised months; overall, the degree of improvement in insulin resistance correlated with weight lost after 29 months. Subjects were then stratified by improvement in insulin resistance. Those with improvement below the median had more severe baseline insulin resistance and higher baseline fasting plasma glucose, as well as a smaller reduction in weight from baseline, whereas those with improvement in insulin resistance above the median continued to lose weight. Multiple linear regression analysis showed that extent of weight loss and baseline fasting plasma glucose independently predicted the percentage reduction in insulin resistance. (McLaughlin et al, 2008)

However, later studies may only add to the confusion. A 2011 study by Hoddy et al, in which obese subjects underwent ADF for eight weeks and were divided into tertiles of baseline insulin resistance, body weight decreased by 4% in each tertile i.e weight loss was unrelated to baseline insulin resistance (Hoddy et al, 2011). Nevertheless, the percentage change in HOMA-IR was significantly different between tertile 1 (where baseline HOMA-IR was within normal range) and tertile 3, suggesting that the relationship is between change in weight and change in insulin resistance. However, in the same year, Mediano et al found that in non-obese females stratified into baseline insulin resistant and non-insulin resistant, CR induced a greater reduction in weight and BMI in those with insulin resistance, effectively demonstrating a relationship between weight loss and baseline insulin resistance (Mediano et al, 2011). However a difference between the two studies was that Mediano et al used non-obese subjects while in Hoddy et al's study the subjects were obese. Similarly, a 2013 study showed that in overweight or obese subjects given six weeks of CR followed by six weeks of weight maintenance and were stratified by weight loss, the group which had the greatest weight loss also had the greatest improvement in insulin sensitivity, effectively confirming Hoddy et al's results. Nevertheless, the extent of weight regain during the weight maintenance period was positively correlated with baseline insulin resistance, echoing Mediano et al's results. (Kong et al, 2013) A recent study showed that fasting can in fact worsen insulin sensitivity in the insulin-sensitive but not in the insulin-resistant, although this was an extremely short-term study with a duration of only 60 hours (Duska et al, 2005).

**Association of time restricted feeding and biochemical risk markers for T2D or metabolic syndrome**

Studies in humans are few but one RCT randomised male athletes to a normal diet or time restricted feeding (TRF), when all calories were consumed in three meals during an eight hour time period (1pm to 8pm), for eight weeks. The results showed that TRF was associated with significantly decreased insulin and glucose from baseline but the change was not different from those on the normal diet. (Moro et al, 2016) The remaining human studies are not RCTs and are mostly investigations of blood glucose during Ramadan, in which all food is consumed in the evening, known as the least healthy time of day in which to eat, and the results are mixed. In rodents, however, it is well known that disruption of the circadian rhythm leads to obesity and metabolic disorders, particularly feeding during the daytime, which is the traditional sleeping time for mice (Yasumoto et al, 2016), but nevertheless, mice with induced obesity, restricted to feeding between eight and 12 hours during the dark (waking) cycle, exhibited reduced weight and fat mass, lowered fasting plasma glucose and insulin as well as improved insulin resistance compared with obese mice with unrestricted feeding, even though the TRF mice were not calorically restricted (Sindaram et al, 2016; Duncan et al, 2016; Hatori et al, 2012; Sherman et al, 2012; Chaix et al, 2014).

**DISCUSSION**

**Findings from RCTs of fasting or CR and biochemical risk markers for T2D**

In subjects without T2D or metabolic syndrome, most trials show that, provided fasting glucose and HbA1c are within the reference range, CR will not lower them significantly, with generally no difference between CCR and ICR. This
reflects the negative feedback mechanism in operation and suggests that CR may have an adaptogenic effect on the body, by lowering metabolic risk factors where it would be of metabolic benefit but having no significant effect where there is no metabolic benefit. In contrast, CR will significantly reduce fasting insulin and insulin resistance, regardless of baseline levels. In subjects with T2D, prediabetes or metabolic syndrome or where mean baseline fasting glucose or HbA1c is elevated or at the upper threshold of the reference range, CR can significantly improve fasting glucose, HbA1c and insulin, insulin resistance and glucose tolerance, causing pre-diabetics to become normo-glycaemic. Studies are divided over whether CCR and ICR are equally effective.

There are also mixed results for trials investigating the effect of different levels of CR, although generally all levels can lower fasting insulin without distinction, provided the study duration is sufficiently long. In subjects without T2D or metabolic syndrome, the CR diet content makes no difference to the results for fasting glucose or HbA1c but in subjects with T2D or metabolic syndrome, benefits are observed with liquid meal replacement and a low carbohydrate, high fat CR. When considering reduction of fasting plasma insulin and insulin resistance, liquid meal replacement and low carbohydrate CR were again beneficial. With respect to supplements, ω-3 PUFAs, vitamin D and L-carnitine can lower risk markers over and above CR. Finally, RCTs of bariatric surgery versus CR show that VLCDs are equally as effective in lowering fasting glucose, insulin and insulin resistance and improving glycaemic control and β-cell function, suggesting that potential surgical patients should first be offered the option to fast.

Findings from non-RCTs investigating the association of T2D risk markers and weight change

Non-RCTs investigating the association of risk markers for T2D and weight change show that a close positive correlation may exist between weight change and fasting HbA1c, with considerably greater weight loss being achieved once normal glycaemia is attained, presumably because glucose-mediated insulin secretion is reduced. Similarly, those achieving the greatest weight loss also achieved the greatest reduction in fasting insulin, while insulin resistance and fasting plasma glucose were independently correlated with weight loss. There are some controversies, however, concerning the association of insulin resistance and weight. Ethnicity appears to have a bearing on this association, with a close correlation in Caucasians but little correlation in African Americans.

Findings from studies of TRF on biochemical risk markers for T2D

There are very few human studies of TRF and results from the one RCT were inconclusive. Nevertheless, studies of mice with induced obesity showed reduced weight and fat mass, as well as significantly lower fasting plasma glucose and insulin and improved glucose tolerance and insulin sensitivity compared to a control group. It is important to note that these results were achieved with no CR whatsoever; both the control and TRF mice ate ad libitum.

It is thought that the development of obesity and T2D may be linked to circadian rhythms, through consumption of food outside the appropriate phase or other disruption such as through shift work, known to be associated with CVD, obesity and T2D, as has been clearly demonstrated in animals. Melkani and Panda suggest that the circadian system is able to sense the metabolic state of the cell, through some as yet unknown signalling mechanism, and it then regulates the timing of expression of a large number of genes based on the information received (Melkani and Panda, 2017). It appears that TRF can restore appropriate rhythmicity partially by interaction and cross-talk between components of the circadian clock and cellular energy signals, and partially through elongation of the daily fasting interval (Antoni et al, 2017; Melkani and Panda, 2017); in the rodent study by Chaix et al, the extent of the metabolic improvements was proportional to the fasting interval (Chaix et al, 2014). Other mechanisms have been suggested by Chaix and Zarrinpar, who proposed that TRF operates by restoring cyclical variation in the elements of the microbiome thought to be involved in metabolism (Chaix and Zarrinpar, 2015). Additionally the microbiota may modify bile acid signalling, implicated in both glucose and energy metabolism.

Towards an optimum regimen to lower biochemical T2D risk markers

As noted above, the pancreas secretes insulin in response to elevated blood glucose concentrations, following digestion. Glucose in the circulation is not necessarily derived directly from sugar in the diet but may be produced from the digestion of all foods, but particularly carbohydrates. It would therefore follow that the only means of lowering both blood glucose and insulin is to reduce and/or delay eating. This can be achieved by fasting, CR or TRF. As has been seen in this review, these methods can reduce fasting insulin and insulin resistance, regardless of baseline levels, and will reduce fasting glucose and HbA1c where they are elevated. Where T2D is diagnosed, only medication or radical dietary intervention such as CR can ensure that blood glucose concentrations do not reach toxic levels, but medication will not be effective in reducing insulin levels and the administration of insulin in advanced T2D will only worsen hyperinsulinaemia and insulin resistance. Only fasting, CR or TRF can lower both fasting blood glucose and insulin.

In diabetics, comparisons of extent of CR show that VLCDs (400-800 kcal/day) are more effective in lowering fasting plasma glucose, and possibly HbA1c, than 1500-1800 kcal/day during the weight loss programme, but results during the weight maintenance period are mixed.
and there may be ethnic differences. An interesting study by Williams et al (Williams et al, 1998) with several different permutations of CR, found that although there was no difference between groups in HbA1c reduction, the greatest number with normalised HbA1c occurred in the VLCD group on a regimen with the greatest number of consecutive fasting days, rather than the group with the greatest extent of CR or the greatest total number of days of CR, a result echoed in rodent studies. Diabetics who attained normal glycaemia had significantly greater weight loss than subjects whose HbA1c level remained >6%.

Studies of CCR vs ICR or ADF generally find no difference in lowering fasting glucose or HbA1c, although ICR appears to be more effective than isocaloric CCR in lowering serum insulin and insulin resistance. These RCTs showing a clear benefit of ICR/ADF over isocaloric CCR indicate that it is not CR alone which makes a successful insulin-lowering regimen; there is some other factor involved, which may be the absence of any glucose-mediated insulin secretion for long periods.

CONCLUSION

In subjects without T2D or metabolic syndrome, and where fasting glucose or HbA1c are already within the reference range, CR will generally not lower them significantly. However, in subjects with T2D or metabolic syndrome or where baseline fasting glucose or HbA1c is elevated, CR can significantly improve these parameters. Nevertheless, fasting insulin and insulin resistance can be significantly improved by CR regardless of baseline levels. Studies are divided over whether CCR and ICR are equally effective or whether ICR can lower T2D biochemical risk markers to a greater extent. There are also mixed results for different levels of CR, with a VLCD possibly reducing fasting glucose and HbA1c to a greater extent than moderate CR, although there was no difference for reduction in fasting insulin. TRF may similarly improve T2D risk markers but this has only been shown in animal studies. Liquid meal replacements, a low carbohydrate CR and vitamin D supplementation may benefit all parameters, while the addition of ω-3 PUFAs and L-carnitine may be of benefit for reducing fasting insulin and insulin resistance. Exercise has no benefit over and above CR.

Although it seems clear that there is a close positive correlation between weight change and HbA1c, there has been some controversy concerning the precise relationship between weight change and insulin resistance, particularly since it was observed that a number of obese subjects may have normal insulin sensitivity while others may be insulin resistant with a normal BMI. The studies may be reconciled to some extent by stratifying by ethnicity; a significant association appears in Caucasians but not in African Americans. There is further controversy over whether the degree of weight loss with CR is related to baseline insulin resistance or reduction in insulin resistance. The most effective regimen to reduce biochemical risk markers for T2D, at least in Caucasians, appears to comprise an ICR programme employing a VLCD with the longest possible number of consecutive days of fasting. This is consistent with the original premise that since glucose-mediated insulin secretion only occurs as a result of eating, both CR and a delay in eating would be of benefit.

This research received no specific grant from any funding agency, commercial or not-for-profit sectors

There are no known conflicts of interest

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Fasting and Caloric Restriction Show Promise for Reducing Type 2 Diabetes Biochemical Risk Markers

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